Behavioural aspects of the New Zealand octopus *Pinnoctopus cordiformis*: acclimation, sleep deprivation and responses to video stimuli

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Picture the scene
where whatever you thought would,
in the blink of an eye,
manifest and become illustrated

You'd be sure, man,
that every line drawn
reflected a life that you loved
not an existence that you hated…

Incubus
Redefine

Acknowledgements

Most importantly, I dedicate this work to my mother Karla, who STILL doesn't see how amazing she is (someone should really tell her).
To my brother Nathan, for growing up so fast without rushing, and for sharing the journey.
To my father, who is always close to my heart, even though we walk in very different worlds.
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Abstract

The native New Zealand octopus *P. cordiformis* is a relatively unstudied member of the cephalopod class. Behavioural investigation will widen the breadth of our knowledge about octopus behaviour away from the handful of classically studied species. Here I test the acclimation patterns of *P. cordiformis* in an experimental environment, using video recording to document and analyse behaviour over a three-day period. I also test *P. cordiformis* following acclimation, examining the role of sleep in mediating behaviour, and test for the presence of homeostatic regulation on behaviour following sleep deprivation. Finally I examine the responses of *P. cordiformis* to video playback, testing the way in which motion and shape mediate predatory behaviour in *P. cordiformis*, and risk assessment in response to a sympatric predator (*Arctocephalus forsteri*).

Pinnoctopus cordiformis responded well to acclimation, with little if any change in behaviour over the three day acclimation period, and minimal indication of stress (e.g., only one case of food rejection, and no changes in groom rates). Acclimation was not influenced by sex differences, or by differences in body weight or limb condition. *Pinnoctopus cordiformis* did not appear to possess homeostatic regulation of sleep behaviour following sleep deprivation, indicating that the variability in sleep and rest patterns seen in aquatic mammals and elsewhere also exists between octopuses of different species. Video playback of prey stimuli elicited context-relevant responses including changes in luring, peering and alertness as well as appearance characteristics, and this response was mediated by both prey motion and shape. Predator playback elicited semi-realistic responses including adjustments in alertness, flinching activity, and strong adjustments in rates of ventilation caused by behavioural freezing, as well as an alternate fight—flight response depending on video realism.

Table of Contents

Acknowledgements ..... **iii**

Abstract ..... **iv**

Chapter 1: General Introduction **1**

Chapter 2: General Methods **10**

Capture and Housing **10**

Feeding and Cleaning **11**

Day-night cycle **12**

Obtaining video data **12**

Recording and quantifying octopus behaviour **13**

1. Activity 14

2. Locomotion 16

3. Aversion 16

4. Appearance 16

Consideration of behaviours in each experiment **17**

Treatment of data 17

Chapter 3. *Pinnoctopus cordiformis* in captivity: Acclimation & observations **19**

Introduction **19**

Methods **20**

Results **21**

Activity 21

Aversion 21

Locomotion 21

Appearance 22

Discussion **27**

Influence of time 27

Influence of sex 28

Influence of limb damage 29

Influence of weight 30

Do octopuses acclimate? **31**

Chapter 4: Sleep Deprivation 35
Introduction 35
Defining sleep 35
Sleep and cephalopods 37
Convergence 38
Methods 39
Results 40
Activity 40
Locomotion 40
Appearance 40
Supplementary Observations 41
Discussion 45
Conclusion and future directions. 49
Chapter 5: Video playback – Response to prey 51
Introduction 51
Methodology 52
Procedures 52
Stimuli 53
Analysis 54
Results 55
Activity 55
Appearance 56
Reactivity 56
Discussion 65
Does *P. cordiformis* respond to video playback? 65
Active forager or passive predator? 66
Mechanics and caveats 68
Conclusion 68
Chapter 6: Video playback – Response to predators 70
Methods 72
Scoring of video playback data 73
Analysis 73
Results 73
Appearance 73
Reactivity 74

Discussion **84**
Conclusions & Future Directions **88**
Chapter 7: General Discussion **89**
Synopsis **89**
Methodological considerations **91**
Conclusion and future directions **92**
References **96**
Appendix 1: Acclimation **125**
Appendix 2: Sleep Deprivation **133**
Appendix 4: Predator Playback **147**

Chapter 1: General Introduction

Octopuses are fast-swimming, predatory cephalopods (Mather, 2008). They are found in marine habitats all over the world, where they occupy benthic and pelagic zones (Hanlon & Hixon, 1980; Anderson, 1999; Wood & Anderson, 2004; Kuba *et al*., 2006). Octopuses forage on a wide variety of prey (Anderson *et al*., 2008), and are renowned for their complex behaviour (Hanlon & Hixon, 1980; Fiorito & Scott, 1992; Messenger, 2001; Barbato *et al*., 2007; Mather, 2008a, Mather 2008b). Cephalopod intelligence is recognised as the highest among all invertebrates, with members of this group documented to possess remarkable spatial memory (e.g. Mather, 1991; Alves *et al*., 2007), conditional discrimination (e.g. Hvorechny *et al*., 2007), and learning ability (e.g. Fiorito & Scotto, 1992). This is facilitated by a highly centralised nervous system and lateral partitioning of brain function (Mather, 2008) that parallels the structure of vertebrate brains. Here I examine several aspects of octopus behaviour in a native New Zealand octopus (*Pinnoctopus cordiformis*), testing behavioural responses to captivity, sleep deprivation, and to video playback stimuli (fig 1.1).

P. cordiformis, also known as *Macroctopus maorum* (Norman & Hochberg, 2005), is found in littoral zones to depths of approximately 300 metres around mainland New Zealand and the Chatham Islands (O'Shea, 1999), as well as southern Australia (Norman & Reid, 2000). This species is the largest octopod in Australasia (Norman & Reid, 2000), and the third largest in the world (Lalas, 2009). It typically inhabits sandy and rocky substrata and forages mainly on arthropods, fish and other molluscs (Anderson, 1999). These octopuses are identifiable by their orange-brown or purple-gray colouration; the presence of iridescent white spots on the arms and web; and the absence of these spots on the mantle (Anderson, 1999). Apart from this information, very little is known about the species.

Fig. 1.1: Male *P. cordiformis* "Outside Kitty" sitting with limbs partially tucked underneath the mantle. Iridescent white spots cover the arms, and the body is most commonly a warm orange-brown colour or a dark purple-gray.

Most octopuses are speculative chemotactic foragers, pouncing on locations that might house potential prey (Chase & Wells, 1986; Forsythe & Hanlon, 1997). They utilise specialised cells near the surface of the skin to alter the appearance of their bodies, which is useful both in capturing prey and avoiding predators such as marine mammals, fish, sea birds and other octopuses (Anderson, 1999; Messenger, 2001; Lalas, 2009; Staudinger *et al*., 2011).

Cephalopod visual systems are highly sophisticated (Messenger *et al*., 1973; Shashar & Cronin, 1996; Mäthger *et al*., 2006; Kelman *et al*., 2008). Their camera-like eyes are analogous to vertebrate eyes (Budelmann, 1994; Ogura *et al*., 2004), capable of high spatial acuity, discriminating shape, contour and pattern (Muntz & Gwyther, 1988; Zylinski, 2009). Though cephalopod eyes are well understood and able to support exceptional visually based behaviour, how the characteristics of octopus vision relate to *in situ* environments where decisions are made about foraging and anti-predatory risk assessment has not been assessed.

There is a strong tendency in the field of octopus behaviour for researchers to focus their efforts on a handful of well-studied species. This has led to a wealth of information based on observations on a relatively limited group of species, such as *Octopus vulgaris* (e.g. Fiorito, Planta & Scotto, 1990; Mather & O'Dor, 1991; Fiorito & Scotto, 1992; Hochner *et al*., 2003; Brown *et al*., 2006; Anderson *et al*., 2008; Gutnick *et al*., 2011)*, O. cyanea* (e.g. Yarnall, 1969; Forsythe & Hanlon, 1997; Mather & Mather, 2004) and *O. bimaculoides* (e.g. Boal *et al*., 2000; Sinn *et al*., 2001; Hvorecny *et al*., 2007). Although much can be assumed about the behaviour of *P. cordiformis* from looking at these species, there are risks in drawing assumptions about unstudied species from those that have been previously studied. Selection pressures that are unique to certain animals can produce species-specific differences that mask taxon-wide traits, making it difficult to establish whether a behaviour or physiological trait is common to a group of animals or only occurs in a smaller subset of that group. It is therefore important, where possible, to include unstudied species for a comparative approach to behaviour. This helps widen the scope of information in the field of interest, and reduces the likelihood of overgeneralising traits that are found in only few species.

In this study, I examine: (1) the acclimation behaviour of *Pinnoctopus cordiformis* to a captive environment; (2) the effects of limiting rest on octopus behaviour and the potential for behavioural 'sleep' in *P. cordiformis*; (3) the responses of this species to video playback of a prey item and the role of stimulus motion and shape in mediating predatory behaviour; and (4) responses to digital animations of a predator in comparison to video stimuli as well as investigate the role of apparent size in the risk assessment in *P. cordiformis*.

Because so little is known about the behaviour of *P. cordiformis* it is important to establish a better understanding about the behaviour of this species in a captive environment. This is the aim of Chapter 3. This follows a description of the general animal maintenance methods in Chapter 2. The observational results from Chapter 3 can act as a platform on which subsequent studies may be based, and at the same time will enable better comparison with other species of octopuses. Traditional techniques developed from research on well-studied animals may not be optimal for animals with differences in their behaviour and physiology. This can compromise the testing of, and caring for, experimental subjects. Anderson & Wood (2001) note that maintenance and experimental methods with different species should take differences in body size and ecological background into account, and so examining *P.*

cordiformis, one of the largest octopuses known (Norman & Reid, 2000; Lalas, 2009), will be valuable for methodological comparison with other octopus species in future studies.

Investigating the way in which *P. cordiformis* individuals adapt and acclimate to an experimental environment is important given that the species has not been examined experimentally. Unacclimated animals have been shown to exhibit higher levels of stress. This may compromise experimental results through unbalanced body chemistry (Young, 1981; Langkilde & Shine 2006), unresponsiveness (Balcombe *et al*., 2004), compromised immune systems (Balcombe *et al*., 2004), aversion to or aggression towards other subjects, experimenters, or themselves (Mason, 1991), and other stereotypies or displacement behaviours (Mason, 1991; Wickens & Heleski 2010). Some octopuses are known to cannibalise conspecifics (Ibàñez & Keyl, 2010), and this can be triggered by stress (Hanlon & Messenger, 1996). It is clear that reducing the effects of stress is paramount to obtaining biologically meaningful results when testing octopuses in the laboratory. The alternative to acclimating wild-caught animals is to rear subjects in captivity, but this is not always viable. Housing animals for their entire lives is costly, and limitations on space and/or funding can sometimes limit studies to only a few individuals. Furthermore, environmental cues are sometimes overlooked in captive environments, and this in turn can compromise the development of the focal animals (Sackett *et al*., 1999; Moberg *et al*., 2011). In the present study I have chosen to study wild-caught *P. cordiformis* for exactly this reason. Little is known about *P. cordiformis*, and even less about their development (Anderson, 1999). The University of Canterbury's Edward Percival field station (Kaikoura, New Zealand), along the shores of the Pacific Ocean, allowed me to conduct studies on live-caught, ocean-reared animals that had developed with the full suite of natural cues. A clear disadvantage to this is that captivity is a comparably unnatural environment for wild octopuses, and this may incur some level of stress. However, the nature of that stress, and how it impacts *P. cordiformis'* behaviour, was one of the main objectives of this study.

In Chapter 4, I examine the behavioural impacts of preventing octopuses from resting. In particular, I examine the potential expression of behavioural 'sleep' in *P. cordiformis*. It is important to distinguish between 'true sleep', which is thought to be relatively rare, and behavioural quiescence, which is common to a vastly greater number of species (Capellini *et al*., 2008; McNamara *et al*., 2009). True sleep is taxonomically widespread, and is observed in birds, mammals, and possibly reptiles (McNamara *et al*., 2009). Some evidence has begun

to emerge that supports the existence of true sleep in a small number of invertebrates, including *Apis mellifera* (Kaiser, 1988; 2002; Sauer *et al*., 2004) and *Drosophila* spp. (Hendricks *et al*., 2000; Shaw *et al*., 2000). There are strict criteria that separate animals engaging in true sleep versus engaging in metabolic or behavioural quiescence regulated by circadian rhythms (Siegel, 2005; McNamara *et al*., 2009). For instance, sleep is homeostatically regulated, and depriving an animal that truly sleeps of opportunities to sleep will generate homeostatic behaviour, or 'sleep rebound' (McNamara *et al*., 2009). For example, depriving rats of sleep increases the amount of sleep behaviour observed after deprivation trials (Suchecki *et al*., 2000), and gives rise to selective increases in brain dopamine metabolism during post-trial REM sleep (Wojcik & Radulovacki, 1981). This observation has resulted in what is referred to as the sleep deprivation or sleep interruption paradigm (Tartar *et al*., 2006) and is used widely to test for the presence of sleep in animals (e.g. Rechtschaffen & Bergmann, 2002; Brown *et al*., 2006; Tartar *et al*., 2006). Recently, Brown *et al*. (2006) used sleep deprivation techniques to show that behavioural quiescence in *Octopus tetricus* had a homeostatically regulated component to it. They also demonstrated an analogy associated with memory processing between *O. tetricus* brain activity and mammalian brain activity. This analogy is remarkable, given the evolutionary distance between mammals and octopuses. Sleep plays a role in memory consolidation and learning in mammals (Hobson, 2005; Walker, 2008a; Walker, 2008b) and has been correlated to brain size, among other variables (Allison & Cicchetti, 1976). Octopuses are renowned for their memory and learning (Mather, 2008) as well as their comparably large brains. Packard (1972) found that the brain-body ratio of cephalopods approaches that of birds and mammals, and exceeds that of most fish and reptiles.

The potential parallel evolution of 'sleep' in octopuses, invertebrates that are well known for their cognitive behaviour, and vertebrates, offers an excellent chance to examine sleep outside of the well-studied arena of vertebrate sleep behaviour (Capellini *et al*., 2008), where the function and evolution of this phenomenon remain contentious and unresolved (Rechtschaffen, 1998; Siegel, 2005; Stickgold, 2005; Tononi and Cirelli, 2006; Lima and Rattenborg, 2007; Rattenborg *et al*., 2007; Lesku *et al*., 2008). Other researchers argue that the role of sleep is not related to brain development but instead evolved solely for metabolic purposes (Berger & Phillips, 1995; see Capellini *et al*., 2008 for review).

While one way in which sleep behaviour might be investigated is through neurological examination, surgical alteration can be restrictive and extremely stressful to animals. Here I make use of sleep interruption paradigms to prevent *P. cordiformis* from sleeping, and study the effects of this interruption on subsequent behaviour to determine whether any form of homeostatic regulation occurs in response to sleep deprivation. This is the second study to test the hypothesis that 'sleep' deprivation in octopuses should cause homeostatic regulation of behaviour, and previous evidence from Brown *et al.* (2006) is encouraging. Helping to expand our knowledge about the nature of rest in octopuses will enable comparisons of cognition and intelligence between cephalopods and higher vertebrates (Mather, 2008), and will help to resolve the contention surrounding the existence of behavioural sleep in invertebrates (McNamara *et al.*, 2009).

Another way in which this study will help to develop our understanding of octopus behaviour is by continuing recent pioneering work in the field of video playback. The development of video playback has enabled researchers to isolate and manipulate visual cues with precision (Oliveira *et al.*, 2000, Bird & Emery, 2008). Previous presentationsof static images presentation often failed to elicit realistic responses (Ryan & Lea, 1994), but video playback has been successful with a range of animals, including birds (e.g. Evans & Marler, 1991; Rieucau & Giraldeau, 2009; Smith & Evans, 2011), reptiles (e.g. Nelson, *et al.*, 2010), fish (e.g. Johnson & Basolo, 2003) apes (e.g. Capitanio, 2002), spiders (e.g. Clark & Uetz, 1990; Pruden & Uetz, 2004) and recently, octopuses (Pronk *et al.*, 2010). Video playback techniques have, for example, allowed experimenters to inspect the role of vision in multimodal signalling, where multiple cues are integrated simultaneously to regulate behavioural responses (e.g. Hansknecht & Burghardt, 2010; Smith & Evans, 2011). The further development of computer-based animation techniques has enabled researchers to generate sophisticated models and manipulate parameters, such as details of a stimulus' shape, size or movement patterns, at unprecedented levels (Nelson & Jackson, 2006; Nelson *et al.*, 2010; Oliviera *et al.*, 2010; Butkowski *et al.*, 2011).

Pronk *et al.* (2010) demonstrated, for the first time, that octopuses (*O. tetricus*) can respond realistically to video playback of a food item, a novel object, and a conspecific. Their study used video playback techniques to interrogate the stability of behavioural suites in *O. tetricus* over time and test for the presence of "personality", which has been used as supplementary evidence for consciousness by other authors (Sinn *et al.*, 2001; Mather, 2008). Evidence from

Pronk *et al*. (2010) suggests that *O. tetricus* does not, in fact, possess a suite of behaviours that is stable over time, but this inspection nonetheless highlights the remarkable scope of questions that can be addressed through the use of video playback in a controlled environment. This is encouraging for video playback studies on cephalopods, enabling researchers to examine a diverse suite of behavioural responses across multiple contexts, including visual communication, learning, social and reproductive behaviour. In this study, I make use of this technology to look at the responses of *P. cordiformis* to a potential predator and also examine the roles of motion (Ewert, 1974; Fleishman, 1986; Schulert & Dicke, 2002) and shape (Ibrahim & Huntingford, 1989; Heath, 1993) in mediating their predatory responses.

Vision is an important factor in recognising potential predators (Bouskila & Blumstein, 1992), prey (Persons & Uetz, 1997), and conspecifics (Ord *et al*., 2002) and for cephalopods this is no exception (Hanlon & Messenger, 1996). Correctly processing information about the source of the stimulus is fundamental to an animal's decisions regarding foraging behaviour, conspecific interactions, and risk assessment (Lima & Dill, 1990; Dall *et al*., 2005; Ferrari & Chivers, 2011). Conversely, incorrect decisions can lead to an absolute loss of fitness via mortality (Abrams, 2000). Often animals have to make decisions with limited information (Sih, 1992; Dall *et al*., 2005) and so base decisions on one or a few available cues (Blanchard, 1997), which in the visual domain may include movement (Ewert, 1974; Fleishman, 1986; Schulert & Dicke, 2002), shadows (Cooper Jr., 2009), depth judgements (Ydenberg & Dill, 1986) and looming (Schiff *et al*., 1962).

The cues mediating octopus predatory behaviour are not well-studied, although it is generally acknowledged that octopuses are strongly reliant on chemotactic foraging, pouncing speculatively on microhabitats which may contain prey (Chase & Wells, 1986; Mather, 1991; Forsythe & Hanlon, 1997). For example, including chemical stimuli in a food puzzle where prey were contained within a jar reduced trial times in *Enteroctopus dofleini* (Anderson & Mather 2010). However, vision plays a strong role in other aspects of cephalopod behaviour, such as learning (Papini & Bitterman, 1991; Fiorito & Scotto, 1992; Mather, 2008b), habituation (Mather & Anderson, 1999; Kuba *et al*., 2006), spatial memory and navigation (Mather, 1991). Pronk *et al*. (2010) argue that cephalopods rely mainly on visual signals when interacting with conspecifics, predators and prey. While chemotactic speculative foraging may be advantageous in situations where prey are obscured by sea-floor fauna, this does not

preclude the use of visual stimuli in mediating predatory behaviour in octopuses. Accordingly, in Chapter 5, I examine the role of motion and shape in mediating predatory behaviour in *P. cordiformis* on a sympatric decapod (*Cancer novaezelandiae*) by utilising video playback technology, which was recently shown to elicit realistic behaviour in another octopus species (*O. tetricus*; Pronk *et al*., 2010). I hypothesise that motion and prey shape will influence behaviour.

P. cordiformis shares its habitat with a number of fast-swimming pelagic predators such as the New Zealand fur seal (*Arctocephalus forsteri*; Anderson, 1999), and has to identify and evade these predators. The antipredatory behavioural suite of octopuses is quite remarkable. They make use of camouflage, chemical deterrents (inking), body-image deception (mimicry), and also use rapid, high-pressure escape-jetting (Forsythe & Hanlon, 1997; King & Adamo, 2006; Hanlon *et al*., 2009). However, the way in which octopuses assess the risk that leads them to perform these escape mechanisms is unknown. Motion (Nakayama & Loomis, 1974; Regan & Beverley, 1984) and looming (Regan & Vincent, 1995; Carlile *et al*., 2006) are known to play an important role in risk-assessment (Schiff, 1965; Nelson *et al*., 2010) and are investigated Chapter 6.

The integration of computer animation and video playback over recent years has enabled manipulation of specific parameters (e.g. morphology, movement; Rosenthal *et al*., 2004; Pruden & Uetz, 2004; Nelson *et al*., 2010) with a remarkable degree of precision. Visually-mediated antipredator responses have been well examined in vertebrates (e.g. Carlile *et al*., 2006; King & Adamo, 2006; Ordiz *et al*., 2011; Smith & Evans, 2011) and some invertebrates (e.g. Nelson & Jackson, 2006), but studies of the role of vision in anti-predatory behaviour in cephalopods are relatively uncommon (Mather, 2008). This is surprising given the complexity and acuity of cephalopod vision (Hamasaki, 1967; Zylinski, 2009; Pronk *et al*., 2010; Tricarico, 2011), and the convergent similarities of the cephalopod eye with the vertebrate eye (Budelmann, 1994; Ogura *et al*., 2004). The study presented in Chapter 6 examines the responses of *P. cordiformis* to computer animations of a sympatric predator (*A. forsteri*) which is seen to loom toward the octopus, recede, or simply move laterally from one side of the screen to another. I test the hypothesis that apparent looming will stimulate stronger antipredatory responses than less threatening stimuli (receding and laterally moving seals). The responses of *P. cordiformis* to these animations is compared with baseline behaviour and also with its responses to archival footage of *A. forsteri*.

Following on from my observational and experimental chapters (Chapters 3-6) I will end my thesis with a general synthesis, discussion and conclusion in Chapter 7, interpreting the results generated and offering direction for future research in light of the findings of these studies.

Chapter 2: General Methods

Capture and housing

Octopuses were delivered to the Edward Percival Field Station, Kaikoura, from local fisherman who removed them from crayfish pots. Upon delivery, each individual was transferred into one of three 71cm x 100cm x 70 cm (Height x Length x Width) acrylic 470L purpose-build aquaria. Each aquarium was enriched with rocks of varying sizes ($10mm^3$ – $300mm^3$), which served as a substrate as well as shelter for occupants. Larger rocks were arranged to form a den into which octopuses could retreat. Anderson & Wood (2001) suggest that dens need only be of slightly greater volume than the occupant, and so dens were constructed specifically to accommodate the size of the octopus inhabiting the tank.
Each aquarium was fed by a hose at the top of the tank which supplied ~10ºC fresh seawater pumped directly from the ocean, which lies adjacent to the Edward Percival Field Station. Water drained from each aquarium through a pipe near its base. Tanks were especially designed to minimise chances of escape. To this extent the tanks incorporated a lid, which was secured to the tank with screws. To allow immediate access to tanks, each lid was constructed with portals that either screwed shut or were twisted before being pulled open.

The condition of each octopus was recorded on arrival. Lesions on the skin were noted, and any damage to arms was recorded as an estimated proportion of limb lost. Immediately after securing the tank lid, the tank was fitted with a trapezoid aluminium frame (Length: 140cm; End 1: 68cm x 95cm; End 2: 20cm x 20cm). The frame was draped with black polythene and hung from the two foremost screws on the tank using looped string (Fig 2.1) . A webcam (Logitech 2.0 MP Webcam C600) was fitted at the end of the aluminium frame furthest from the tank. In addition, the aluminium frames (hereafter referred to as a ‘viewing shields') prevented octopuses from seeing people in the aquarium room from the front of the tank (except through a small gap where the webcams were placed). Experimenter presence has been a factor commonly associated with confounded results (Boycott, 1954; Fiorito *et al.* 1990), so measures were taken to minimise experimenter-subject interactions during trials. To further reduce interactions between visitors and the octopuses, the sides of each tank were covered with black polythene, and signs were posted at each entrance of the aquarium

restricting access exclusively to experimenters. All animal manipulations described within the thesis were approved by the University of Canterbury Animal Ethics Committee.

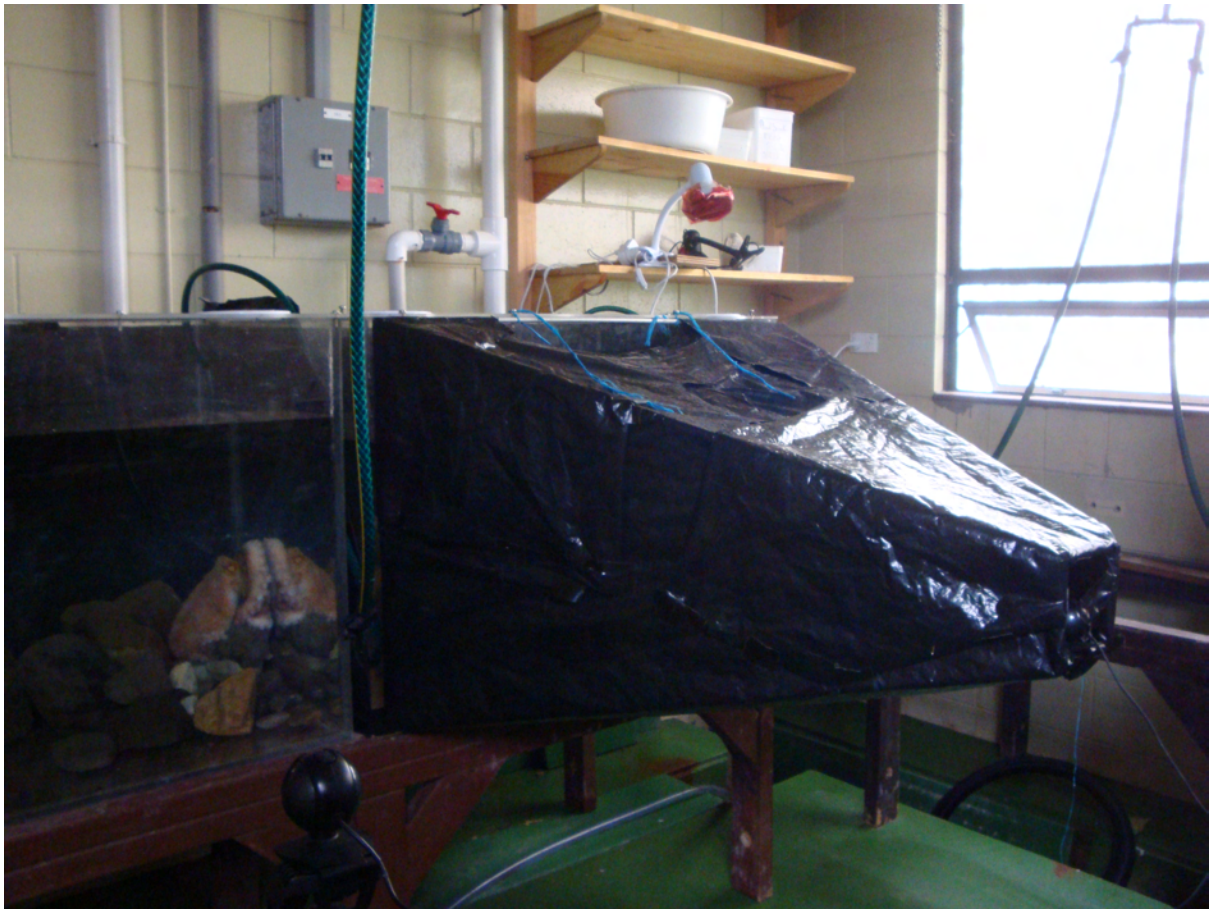

Fig. 2.1: Trapezoid viewing shields were attached to aquaria allowing web-cameras to be mounted for recording *P. cordiformis* behaviours.

Feeding and Cleaning

Animals were fed around dusk every second day. Feeding activity was recorded throughout captivity, rejected food was used as a proximate measure of stress. Prey items included commercially-supplied shrimp, cod, and terakihi, euthanised crabs, and most commonly, salmon acquired from a commercial source. No differences in behaviour or activity levels were observed between octopuses that were fed crabs (*Cancer novaezelandiae*) and those that were not, although this was not formally tested.

The cleanliness of aquaria was closely monitored with twice-daily checks. If food remained in the tank for more than 24 hours it was removed and the tank was cleaned. Otherwise tanks were cleaned once every two days. Where possible, cleaning was timed to minimise stress during trial periods by performing cleaning outside of behavioural assessment. To clean the tanks while octopuses occupied them, the roof of the tank was removed and the water was sifted with a fine mesh plankton net to collect most of the detritus from the tank. Octopuses would typically avoid the net, but some individuals would occasionally attack the net. When attacked, the net was held still until the octopus released its grasp.

More thorough cleaning of the tanks was performed between the departure of one octopus and the arrival of the next. Tanks were drained to 1/3 full and all rocks were removed from the tank. Detritus was collected with a fine mesh plankton net, and each tank was then drained of remaining water. Tanks were then scrubbed and rinsed with hot freshwater, wiped dry and filled with seawater before being drained and filled again with seawater. Rocks were scrubbed and rinsed before being placed into each tank between the first and second seawater fill, and used to form the aquarium substrate and den.

Day-night cycle

Octopuses were subject to a natural day-night light cycle provided from lighting through two windows in the aquarium. Measurement of luminosity using a lux meter indicated that changes in light levels at the ceiling pane of each aquarium tracked changes in outside ambient light levels, and neither light level nor fluctuations across the day differed between tanks.

Obtaining video data

To allow quantification of behavioural patterns during acclimation, sleep deprivation and video playback trials, data were extracted from video footage of each animal taken during its time in captivity. When a new octopus arrived and was introduced to an aquarium, that aquarium was fitted with a viewing shield (see Fig. 2.1), which allowed a webcam to be placed far enough away to capture video of the entire aquarium. Once the viewing shield and camera were in place, recording was started. Recording was managed through Debut Video Capture Software at an average of 10 frames per second at a resolution of 1200x800 pixels

due to processor load. Because Debut can only record through one device at a time, it was necessary to stagger recording when obtaining behaviour samples from multiple octopuses sequentially. Because each aquarium could be fitted with its own viewing shield and webcam, I was able to largely automate video recording of multiple octopuses. To reduce visits to the aquarium, which can compromise behavioural experiments (Fioito *et al*., 1990) and allow filming for long periods during acclimation and sleep deprivation studies, Debut was operated by a macro written in an external program 'Workspace Macro'. Workspace Macro was programmed to access Debut options settings, set the camera to the next webcam in queue, set the appropriate settings (1200x800 resolution, rgb colour settings), activate filming and deactivate filming 10 minutes later, before repeating the process with the next webcam. Filming at night was facilitated by a 75 watt bulb in a portable lamp placed atop the tank and filtered through ~2mm of red cellophane. Octopuses are generally considered to be colour-blind (Messenger, 1973), and have reduced sensitivity to red light, thus although they are not completely insensitive to this light, light levels in this study were deemed sufficient for the purpose of filming without disturbing subjects (Jennifer Mather, personal communication). Once video footage was obtained it was backed up onto an external hard drive and transferred to a MacBook Pro laptop for scoring.

Recording and quantifying octopus behaviour

To provide consistent recording and quantification of behaviours by the observer of each video sample, behaviours were defined during pilot trials. Over the duration of this study it became clear that additional behaviours should be considered, so definitions for these were developed *post-hoc*. Recorded behaviours fell into the wider categories of 'activity', 'locomotion', and 'aversive' behaviours, while 'appearance' characteristics were also monitored and recorded. The behaviours comprising each of these categories are discussed below. Some behaviours were of interest only within the context of specific studies (for example, ventilation rates were only recorded during video playback trials) and thus were not scored outside of these studies.

1. Activity

1. Luring
   Slight movement of only <10cm of one whole arm or slight movement of the distal halves of up to 3 arms.
2. Alertness
   Eyes are completely open, and sometimes distended (see Fig. 2.2).
3. Resting
   Eyes are partially closed but pupil is still visible through a gap in the eyelids. This state was not utilised in analysis, but it enabled discrimination between individuals which were typically less attentive than "alert" octopuses, and not so inattentive that they were thought to be sleeping.
4. Sleeping
   Eyes are completely closed and not distended. 'Sleep' was often accompanied by the obscuration of the eye by folds of skin, but this was not observed every time (see Figs. 2.3 & 2.4).
5. Out of Sight
   Eyes are not visible from the observer's perspective. Although not analysed, this state was used to allow rates of ventilation to be calculated correctly (see Chapters 5 & 6).
6. Handling
   Directly manipulating an object (e.g., rock or pipe), or moving more than 3 arms across an object or substrate.
7. Peering
   Raising the eyes upward away from the body faster than the body is raised.
8. Ventilation
   One cycle of expansion and contraction of the mantle.

Fig. 2.2: 'Alert' octopus eye, where the eye is relatively wide open and skin is clear of the eye.

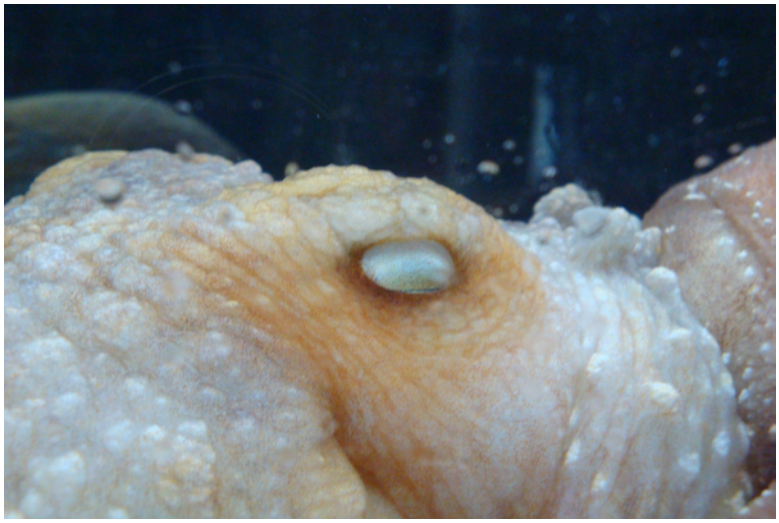

Fig. 2.3: 'Sleeping' octopus eye when closed. Note that the skin is still clear of the eye.

Fig. 2.4: 'Sleeping' octopus eye when closed. In this image the eye is obscured by skin folds.

2. Locomotion

1. Walking
   Four or more arms using suction on the horizontal substrates (rocks & aquarium floor) to draw the mantle in a particular direction.
2. Climbing
   Four or more arms using suction on the vertical substrate to draw the mantle in a particular direction.
3. Swimming
   Expelling water from the mantle cavity through the siphon to generate movement without the use of arm suction.

3. Aversion

1. Grooming
   Arms are moved recursively across the mantle, web and other arms.
2. Hiding
   More than 50% of the octopus is inside the aquarium den.
3. Flinching
   Lowering the eyes toward the body faster than the body is lowered.

4. Appearance

1. Colour
   The most commonly observed colouration (also referred to as 'baseline colour') during pilot trials was an orange colouration evenly across body and arms (see Fig. 2.1). However, colour varied from having the entire mantle, web and arms all white, to all being a dark red/brown colour. This was divided into 5 categorical steps with 1 being lightest and 5 being darkest.
2. Texture
   Absence (referred to as 'smooth texture') or presence (also referred to as "mottled texture") of the expression of sensory statocysts on the mantle cavity.
3. Pattern
   Patches (or absence of patches) of colour that disrupt the overall colour of the mantle and arms. Three categories were of pattern were defined, (1) "bland", where disruptive colouration was absent, (2) "splotch", where disruptive

colouration occurred in large concentrated patches and (3) “sand”, where disruptive colouration patches were small and diffuse.

Consideration of behaviours in each experiment

The first experiment in this study looked at overall behavioural patterns in *P. cordiformis* and thus considers the majority of the behaviours listed above. The exceptions were flinching, peering, and sleeping. The second experiment examines the influence of sleep deprivation on *P. cordiformis* behaviour, and so sleep activity is recorded in addition to the behaviours examined in acclimation. Because sleep behaviour is not independent with respect to alertness, alertness was not recording during sleep deprivation trials. Resting was not utilised in analysis, but was essentially used to encode times when octopuses were not strictly “alert” nor sleeping. Similarly, because states of colour, pattern and texture were not independent from the other states, only one state from each category was used in comparative analysis. The states chosen were considered to be the most common or ‘baseline’ appearances from each category, which were ‘3’ (baseline colour) in the 1-5 colouration scale, smooth texture, and bland patterning.

Experiments three and four examine the responses of *P. cordiformis* to video playback of prey and predators, and only consider a subset of the behaviours listed above. Unlike the acclimation and sleep deprivation experiments, both video playback experiments record the rates of ventilation of octopuses. They also examine luring activity, levels of alertness, and all three appearance characteristics. Furthermore, prey playback trials also consider peering behaviour in response to prey stimuli, while predator playback trials consider flinching behaviour in response to predatory stimuli, neither of which are considered elsewhere.

Treatment of data

The majority of the behaviours examined in these trials were initially recorded in milliseconds as continuous duration data. Peering, flinching, and ventilation were exceptions to this, and were recorded as discrete events (count data). To allow the inclusion of duration data obtained from observation periods that differed in length (for example when technical errors shortened a video sample, or when trial length differed between videos during playback trials), These data were converted into proportion data. Ventilation was also recalculated as proportion data

to provide measurement of the rates of ventilation observed during trials. To allow for comparison between ventilation rates of animals where ventilation was obscured, ventilation rates were extrapolated by first deducting the amount of time in which ventilation was unobservable, then converting breath counts into a per-second measurement. Ventilation counts were recalculated as per-second rates.

Because video samples during acclimation and sleep deprivation trials were separated into 1-minute bins of 10-minute samples, these data was averaged to obtain a mean or median (non-parametric statistics were used extensively in this study), and pooled together where necessary (see Chapters 3-6). Data were also pooled together to allow for testing of variation between different groups of octopuses such as male *vs.* female comparisons, and comparisons between different levels of weight and limb damage.

Where significances were found during Kruskal-Wallis ANOVA tests, further testing of pair-wise comparisons were chosen based on results from Dunns post-hoc testing. This prevented unnecessary testing of the numerous non-significant pair-wise comparisons and reduced data cluttering in results sections. Where significant pairwise comparisons are stated in the results sections, these have come from Kruskal-Wallis ANOVA shown in tables (see appendices).

Categorising octopuses: Weight, damage and sex

Octopus weight, condition (damage score) and sex were recorded in each animal to allow investigation of the influence of each of these traits on behaviour. Weight was measured as wet weight in kilograms. Octopus weight was divided into three categories; lightweight (W1; 0.0-1.9kg), medium-weight (W2; 2.0-3.9kg), and heavyweight (W3; ≥4.0kg). Octopuses were sexed by noting the presence of a hectocotyl on the third right fore-arm of the animal (Anderson, 1999) and recorded either as male or female. No subjects were observed with this arm missing, allowing for sex determination for all animals used in the study. Damage was recorded for each limb as the proportion of that limb which was missing, and summed to provide an overall condition score which ranged from 0-8. Damage was divided into three categories according to this condition score: undamaged (D1; 0), moderately damaged (D2; 0.1-1.49), and heavily damaged (D3; ≥1.50).

Chapter 3. *Pinnoctopus cordiformis* in captivity: Acclimation & observations

Introduction

Focussing research efforts on previously studied species offers the advantages of a wide base of prior knowledge, but can be counter-productive when this knowledge base is applied to congeners from different evolutionary and ecological backgrounds. Differences in the behaviour and physiology of poorly studied animals relative to those that are better characterised can render traditional techniques ineffective, and could compromise the testing of, and caring for, experimental subjects. Within teuthology it is particularly common for studies to focus on a handful of key species, such as *Octopus vulgaris* (e.g. Fiorito *et al*., 1990; Mather & O'Dor, 1991; Fiorito & Scotto, 1992; Hochner *et al*., 2003; Brown *et al*., 2006; Anderson*et al*., 2008; Gutnick *et al*., 2011), *O. cyanea* (e.g. Yarnall, 1969; Forsythe & Hanlon, 1997; Mather & Mather, 2004) and *O. bimaculoides* (e.g. Boal *et al*., 2000; Sinn *et al*., 2001; Hvorecny *et al*., 2007) from which the scientific template for our understanding about octopods is established. This study examines a New Zealand native octopus to the behavioural literature on octopuses. Specifically this chapter assesses the acclimation of *Pinnoctopus cordiformis* to a captive environment for the first time. It also gathers information regarding the basic behaviour of this species, acting as a baseline for other trials in this thesis (see Chapters 4, 5 & 6). This will then serve as a knowledge platform on which other behaviour studies for this species can be based, and as a point of comparison for species differences within the diverse array of octopuses found in the aquatic biosphere.

Maintenance and experimental methods with different species should account for differences in body size and ecological background (Anderson & Wood, 2001). For example, *P. cordiformis* is a diurnal, fast-swimming littoral search predator, and persists in colder waters than the more commonly studied temperate octopuses like *O. vulgaris* and *O. bimaculoides*. As is common in cold-water species (Wood & O'Dor, 2000), *P. cordiformis* is notably larger than more temperate species. In practice the study of animal behaviour is difficult without a robust baseline of practical knowledge about species husbandry, forcing methodology to be extrapolated from studies on other species. As *P. cordiformis* is a species with distinct

ecological and morphological characteristics, it will be helpful to assess its acclimation to laboratory conditions, and to provide a framework for the species' behaviour.

Acclimation to captivity can be critical in obtaining scientifically valid results. Non-acclimated animals may exhibit stress and may therefore perform poorly in cognitive tasks. Stress can result in: unbalanced body chemistry, unresponsiveness, compromised immune systems, aggression towards or aversion to other subjects, experimenters, or themselves and other stereotypies or displacement behaviours (Young, 1981; Mason, 1991; Balcombe, Barnard & Sandusky, 2004; Langkilde & Shine 2006; Wickens & Heleski 2010). Displacement behaviours can greatly degrade the quality of life for experimental subjects (Mason, 1991; Wickens & Heleski 2010), which must be preserved from both the standpoints of ethics and scientific inquiry (Langkilde & Shine, 2006). The alternative to acclimating wild-caught animals is to rear subjects in captivity, but this is not always viable. Housing animals for their entire lives is costly, and can limit the studies to a small handful of animals due to space or funding limitations. Ontogenetic cues may also be absent from rudimentary (or even strongly enriched) environments, which can compromise subject development (Sackett, Novak & Kroeker 1999; Moberg et al. 2011).

The present study takes an observation-based approach to test the feasibility of keeping wild-caught *P. cordiformis* in captivity. It aims to establish baseline behaviour patterns for *P. cordiformis*, examining 1. activity, 2. locomotion, 3. aversive behaviour, and 4. appearance characteristics. Each of these four categories reflects a composite of several behaviours defined in Chapter 2. Here I examine the influence of 1. the amount of time in captivity, 2. sex, 3. body weight, and 4. physical condition on these behaviours in the context of acclimation to captivity.

Methods

Octopuses were captured and housed using the procedures discussed in Chapter 2. Immediately after being introduced into an aquarium, individuals were filmed to obtain video data of their acclimation to captivity. Pilot trials determined that the acclimation period for individuals lasted approximately 72 hours, although this was highly variable. Octopus activity frequently normalised in less than 72 hours, and sometimes did not appear to change in

behaviour at all, so 72 hours was used as a flexible standard, using the acceptance of food as an additional indicator of each octopus's acclimation to their environment. Food was only rejected in one instance, but acceptance began within 72 hours, so it was not necessary to extend the acclimation. Animals in this study were filmed for 24 hours per day for 72 hours, or until acclimation was deemed complete.

Filming of each acclimating individual generated raw footage from which data were collected and pooled. Video from all individuals scored for the behaviours discussed in Chapter 2 was pooled across days, or by sex, weight category or limb condition.

Data consisted of count and proportion data, and were analysed and graphed in Prism 5.0a for Mac OS X. Because data were non-parametric they were analysed using Kruskal-Wallis tests and Mann-Whitney U tests. Where multiple comparisons were necessary, Bonferroni corrections were made to compensate for the inflated possibility of Type I errors.

Results

Activity

Time in captivity did not significantly influence any behaviour relating to activity levels, with differences between days for alertness, handling, and luring being non-significant (Fig. 3.1a-c; see tables 1.1 for test statistics). Similarly, no effect of sex, weight or damage differences were observed for alertness, handling and luring activity (see tables 1.1-1.2).

Aversion

Aversive behaviours were not seen to differ during this study, with respect to any variable. Neither time in captivity, sex, damage, nor weight influenced aversive behaviours, with no observable differences between days for hiding or grooming (Fig. 3.2a-b; see tables 1.3-1.4).

Locomotion

Walking, climbing and swimming (Figs. 3.3a-c) did not differ over time in *P. cordiformis* (see table 1.5). The expression of these activities did not differ between sex, weight or damage categories (see tables 1.5-1.6).

Appearance

Neither time **(**Fig 3.4a-c), sex, weight or damage level significantly affected colour expression, body pattern or texture (see tables 1.7-1.8) in *P. cordiformis*.

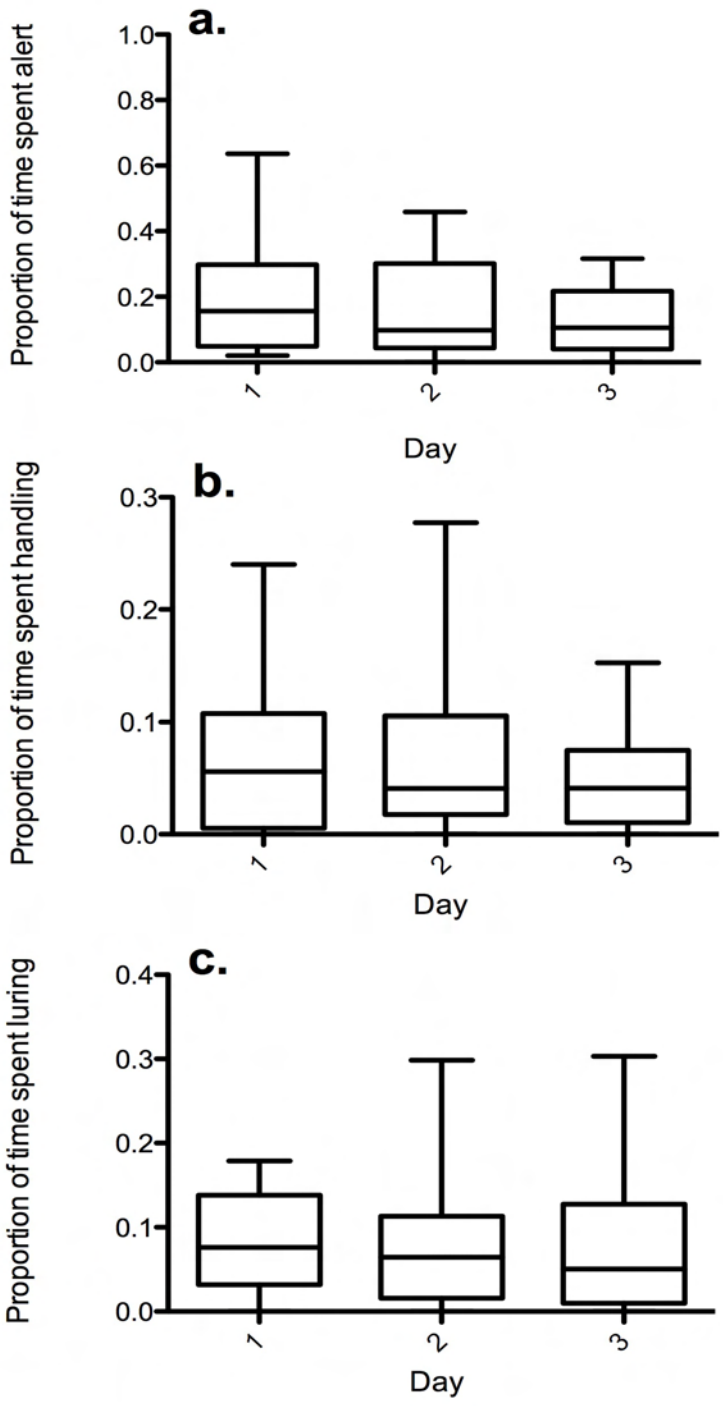

Figure 3.1: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent by *P. cordiformis* engaged in activity behaviours across 3 days of acclimation (n = 16); (a) alertness (b) handling, (c) luring (Kruskal-Wallis ANOVA).

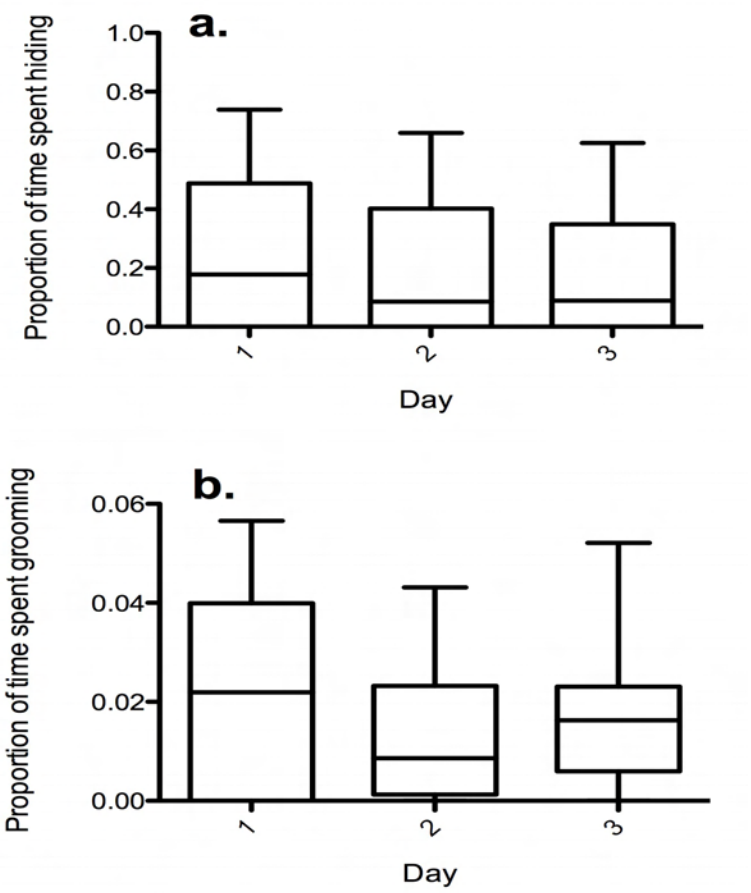

Figure 3.2: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent by *P. cordiformis* engaged in aversive behaviours across 3 days of acclimation (n = 16); (a) hiding (b) grooming (Kruskal-Wallis ANOVA).

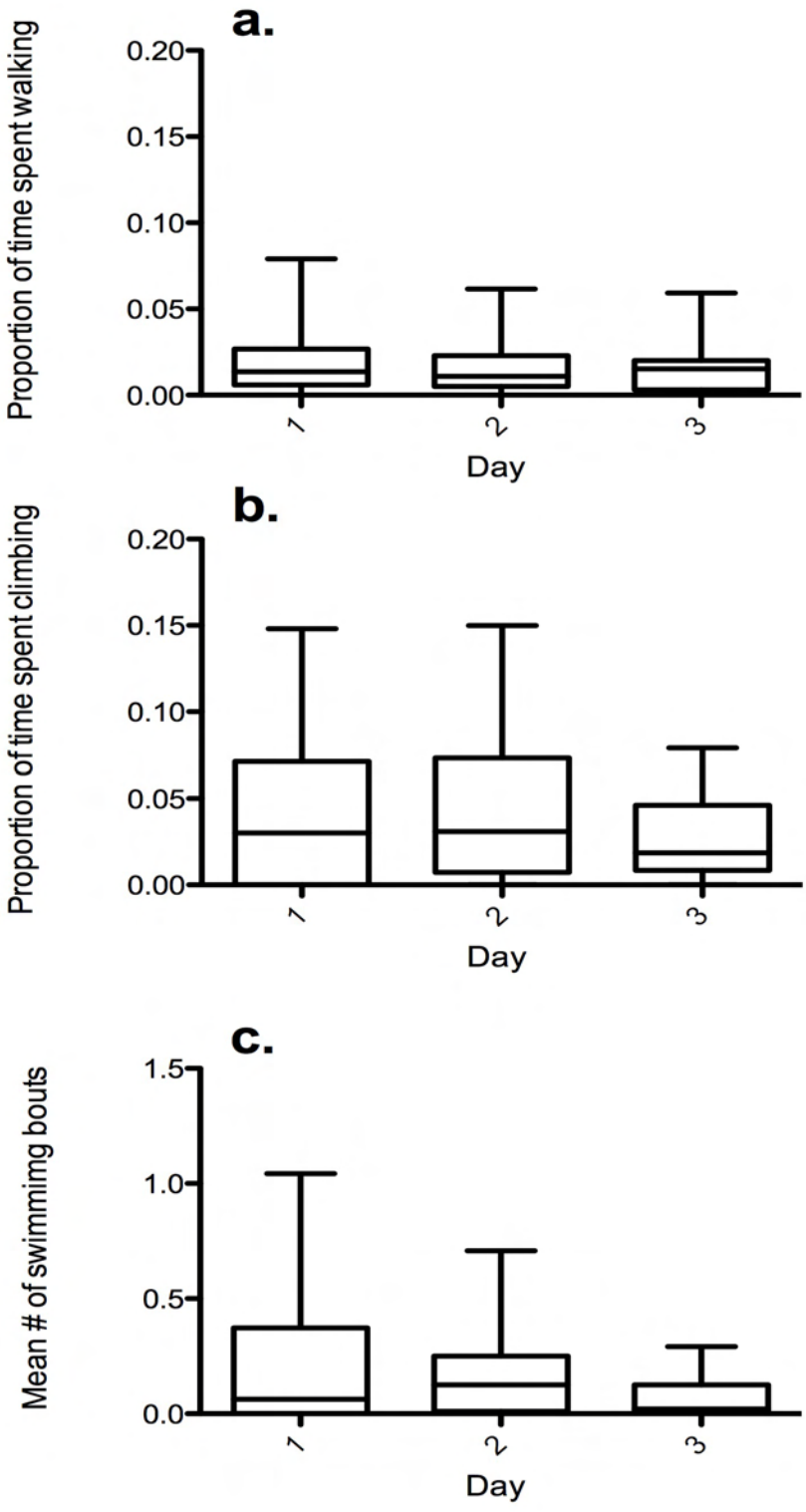

Figure 3.3: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent by *P. cordiformis* engaged in locomotion across 3 days of acclimation (n = 16); (a) walking (b) climbing, and the mean number of swimming bouts across 3 days of acclimation (n = 16; Kruskal-Wallis ANOVA).

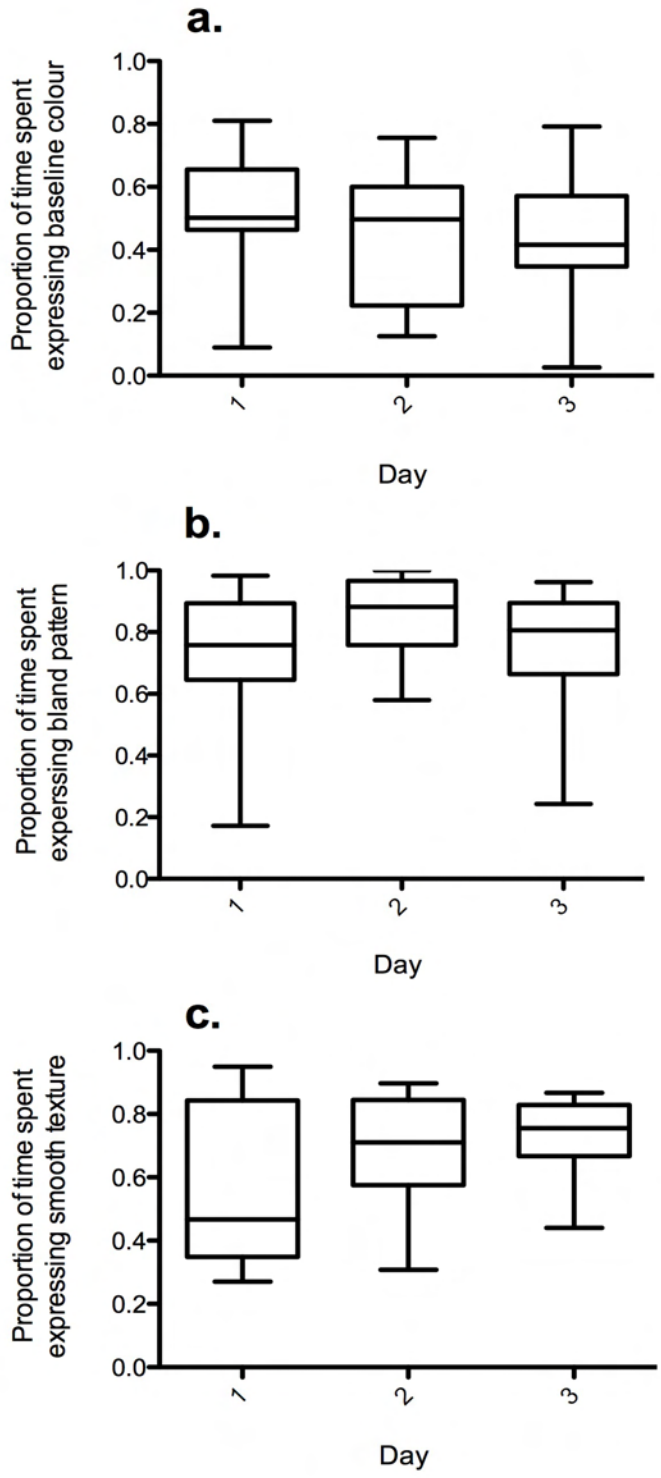

Figure 3.4: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent by *P. cordiformis* expressing appearance characteristics across 3 days of acclimation (n = 16); (a) colour (b) pattern, (c) texture (Kruskal-Wallis ANOVA).

Discussion

Acclimation is often used to allow physiological and behavioural parameters to normalise after stressful interactions, such as capture and handling (e.g. Young, 1981; Langkidle & Shine, 2006; King & Adamo, 2006; Kuba *et al*., 2006). Clear patterns of baseline activity might be expected to emerge during acclimation to a new environment as behavioural and physiological parameters normalise (Balcombe *et al*., 2004). However, the overall lack of change during the three day "acclimation period" of *P. cordiformis* in this study is open to interpretation. On the one hand, the lack of change in baseline behaviour over time may reflect a lack of acclimation to the aquarium environment, while on the other hand it may demonstrate a resilience to stress that could be advantageous for experimental inquiry. Given the evidence discussed below, the latter of these possibilities appeals as a more likely explanation.

Influence of time

Capture and transportation can often be a stressful experience (Grandin, 1997; Adenkola & Ayo, 2010), and it is important that subjects are allowed adequate time for stress levels to normalise before behavioural trials. Often animals are not given a sufficient length of time to acclimate to captivity, and this may compromise performance (Mason, 1991). The amount of time it takes for an animal to resume base-line behaviour (if indeed it resumes at all) varies between species, sex, age, physiological states (see Balcome *et al*., 2004 for examples), and may also be dependent on the environment in which the animal is placed (Carducci & Jakob, 2000). In this study it was interesting to observe that the number of days in acclimation had no influence on the behaviour of *P. cordiformis* in terms of activity behaviour, locomotion, appearance or aversive behaviour. This lack of variation in behaviour in response to time in captivity supports the possibility that *P. cordiformis* shows stable behavioural characteristics in short-term captive environments.

Octopuses are natural explorers (Mather & Anderson, 2007). Learning and exploration play an important role in octopus behaviour (Mather & O'Dor, 1991; Hanlon & Messenger, 1996; Mather 2008b). Thus we might expect that after introduction to a new environment, exploratory behaviour such as locomotion and handling would rise and then decline, yet this was not observed. Octopuses may attempt to minimise foraging time (mainly speculative

chemotactile exploration; Forsythe & Hanlon, 1997) in response to predation pressure (Mather & O'Dor, 1991), and in this case *P. cordiformis* may have reduced its exploratory behaviour in response to the perceived threat of capture and translocation (Balcombe *et al*., 2004). However, exploratory behaviours were constant across days. This suggests that threat-induced behavioural adjustments do not explain the lack of change over time during captivity. A more likely explanation for these observations is the fact that aquaria used in this study were much smaller than the foraging ranges reported in other octopus species. *O. cyanea* has been observed to have foraging ranges as large as $2000m^2$ (500-750g animals; Forsythe & Hanlon, 1997). Foraging ranges in smaller *O. cyanea* of 100-200m (Mather, 1988), suggest that given the large size of *P. cordiformis* individuals, foraging ranges for subjects in this study are likely to be at least comparable, if not significantly greater than the $2000m^2$ range discussed in Forsythe & Hanlon (1997). A temporary home-range of $<2m^2$ as used in this study may limit the amount of exploration necessary in my subjects, and explain the lack of change across days, as a full exploration of the tank might take only minutes.

Influence of sex

Cognitive (Jozet-Alves *et al*., 2008) and morphological (Voight, 1995) sex differences have been previously seen to influence cephalopod behaviour, yet sex did not influence any of the behaviours examined in this study. While it is quite possible that the behavioural parameters studied are not influenced by sex, it may be that behavioural differences might still be found in octopuses outside the age bracket observed in this study. For example, Jozet-Alves *et al*. (2008) found that sexually mature male *Sepia officinalis* travel greater distances and are more likely to use visual cues than females and sexually immature males. Cephalopod learning has a strong ontogenetic component (Mather, 2008), and the possibility that sex-specific behavioural differences are more strongly observable at larval and/or juvenile stages of development cannot be excluded given the results from the present study.

Similarly it is possible that behavioural differences between male and female *P. cordiformis* exist only in sexually active individuals. During the study no gravid females were observed, and so gravidity and incubation are unlikely to have influenced these results. However, it is possible that females may have been fertilised prior to capture and delayed egg-laying and incubation due to stress. Nevertheless, the lack of other stress-associated behaviours does not support this hypothesis.

Influence of limb damage

Octopuses are known to become easily stressed (Malham *et al.*, 2002), but these results suggest that *P. cordiformis* are relatively stress-resilient. Counter to the expectation that stressed octopuses should express a greater amount of stress-associated behaviour, in this study more heavily damaged octopuses did not display greater aversive behaviour (hiding and grooming) than less heavily damaged octopuses. Similarly, no differences were found in foraging behaviours (luring and arm movement) from any damage category. This suggests that more heavily damaged octopuses do not necessarily suffer from a reduction of appetite due to stress (supported by low food-rejection rates in heavily damaged octopuses), nor do they need to offset metabolic demands imposed by the regeneration of lost or damaged limbs (Maginnis, 2006).

Limb damage may cause sufficient stress to induce changes in behavioural patterns (Maginnis, 2006) but stress incurred from a mutilation event may fall rapidly, or *P. cordiformis* may simply be resilient to stress in this form. Of the 25 animals observed during this study, seven had lost ≥80% of an entire arm, and 12 had suffered mutilation of at least half a arm at the time of capture. In other words, limb-loss is a fairly regular event. The loss of an appendage in exchange for survival is, after all, adaptive when the alternative is death, although autotomy does incur metabolic costs and can lower both the reproductive success and survival rates of animals in future predation encounters (Maginnis, 2006). If mutilation is common in *P. cordiformis*, these animals may possess physiological or even psychological mechanisms to diminish stress from limb-loss, with flow-on consequences in terms of robust behavioural responses in the face of physical trauma.

It is important to consider the question ‘is it appropriate to use a chronic condition such as mutilation as a proximate indicator of stress?’. Given that cephalopod behaviour can be strongly ontogenetic (Mather, 2008), it may be that behavioural consequences only arise from predation events occurring before a critical age. Other animals are known to adjust their brain chemistry (sometimes radically) in response to chronic stress and/or strong short-term stress (Katz & Roth, 1979; Katz, 1982; Hart, 1988). Given their strong plasticity (Hanlon *et al.*, 1999; Hochner *et al.*, 2006) and variable environment (Anderson *et al.*, 2008; Sinn *et al.*, 2010), the same might be expected in octopuses, which might lead to misleading assumptions.

While there is no way to evaluate when an appendage was mutilated, mutilations may occur within a single predation event (short-term severe stress) or may occur over multiple predation events (recurring moderate stress). In these subjects some mutilated arms, but not others, appeared to be regenerating (seen as dwarf appendages growing at arm tips), suggesting that predation or other events leading to damage events may have been temporally discrete. Some female octopus species retain the male reproductive appendage (hectocotylus) after reproduction, delaying fertilisation (Boyle & Rodhouse, 2005). It is unknown whether this behaviour is observed in *P. cordiformis,* but removal of the hectocotylus from a male may not alter behaviour in the same way as removal of an appendage through predation. This may add variation to analyses of the influence of damage on behaviour.

Influence of weight

Differences in locomotory and exploratory behaviour might be expected in octopuses of different weights, as individuals may be driven by stronger motivation for food. Low-weight octopuses should require more energy to reach their maximum growth potential than heavier octopuses, but octopus growth is thought to be strongly variable, both between populations and over time in individuals (e.g. Leporati *et al*., 2008). However, this is not supported by the lack of differences in luring behaviour, which here is interpreted as an attempt to capture prey. Furthermore, octopuses exhibit both sit-and-wait predatory behaviour and active foraging (Yarnall, 1969; Mather & O'Dor, 1991), and non-significant differences in locomotory behaviour between octopuses of different weight categories also suggests that low-weight octopuses were not driven to forage proportionately more than heavier individuals.

Octopuses range widely in the wild, with foraging distances increasing with weight (Mather, 1988; Forsythe & Hanlon, 1997). The lack of availability of space within aquariums may limit the scope for octopuses to perform regular foraging behaviour. This should be especially influential in larger octopuses, for whom spatial restrictions are relatively greater. As Rehling (2000) notes, enclosure size can serve as a form of enrichment, increasing activity with larger enclosures. Large octopuses might therefore be expected to be more stressed, but no differences in the expression of stress-related behaviours were observed between weights.

While it is tempting to draw assumptions about octopus age from wet weight measurements, interpretations from these data must be made with some caution. Octopuses can sustain significant damage during their lives, and mutilation of arms can remove much of the body weight of an individual, compromising the relationship between body weight and age. Female wet-weight measurements may be confounded by females which have mated recently prior to capture, retaining hectocotyl or incubating eggs. Older octopuses are more likely to experience predation encounters, resulting in grouping of older damaged individuals together with younger undamaged individuals. A more appropriate solution to this might be to use mantle length or inter-ocular distances when using body parameters as a proximate measure of age, but high variability in octopus growth rates (Semmens *et al*., 2004) diminish the precision of these measurements. Measurement of two sections of the octopus beak (the rostral sagittal sections and lateral wall surfaces; Perales-Raya *et al*., 2010) are considered the most accurate means of age estimation, but these measurements are post-mortem comparisons unavailable to me, as my tests were only based on capture-release of live animals.

The lack of behavioural differences between octopuses of different weight categories suggests that behavioural patterns are stable between octopuses of different ages. This seems counterintuitive, as cephalopods are thought to have a strongly ontogenetic component to their learning and development (Messenger, 1973; Sinn *et al.* 2001; Mather, 2008b). However, Kuba *et al*. (2006), found that post-exploratory play behaviour did not differ between young and old *O. vulgaris*, suggesting that such differences should not necessarily be reflected in general behaviour patterns such as those investigated here.

Do octopuses acclimate?

Animals that do not acclimate to their new environment may suffer physically and mentally, compromising cognitive performance and confounding physiological and behavioural studies (Young, 1981; Mason, 1991; Balcombe *et al*., 2004; Langkilde & Shine 2006). Furthermore, displacement behaviours such as stereotypies (Mason, 1991; Wickens & Heleski, 2006) and autophagy (Maginnis, 2006) can seriously reduce the quality of life for animals. *P. cordiformis* appear not to be strongly stressed by displacement from the wild into a captive habitat. Nevertheless, a number of caveats should be considered before arriving at this conclusion. For example, individual octopuses may respond differently to acclimation as a

consequence of their 'temperament' (suite of correlated behaviours). Cephalopods have been suggested to possess temperament that differs between individuals, but is variable over time (Mather & Anderson, 1993; Sinn, 2001; Pronk *et al*., 2010).

Pre-existing variability in stress levels is a likely contributor to behavioural variability seen in this study, and is an important consideration for future acclimation studies. Without measurement of physiological stress indicators it is difficult to conclusively show that acclimation has occurred, but behavioural evidence including rates of food rejection, stable expression of stress-related behaviour, and an overall lack of behavioural variation between days as trials continued suggests that *P. cordiformis* acclimate rapidly to captivity. However, food rejection may only occur when stress levels become extreme. It is, however, known that stress increases the levels of cocaine- and amphetamine-regulated transcript (at least in rat; Xu *et al*., 2010), a factor known to reduce feed intake in fish (Kehoe and Volkoff, 2008). If a similar effect occurs in octopus then it suggests that food acceptance might be a good measure of acclimation. To my knowledge no studies have addressed the correlation between food rejection and stress in octopuses, which seems a good avenue for further research on cephalopod husbandry.

It is also possible that senescence following mating or hatching eggs prior to capture may have influenced behaviour of either sex, but the signs of deterioration are clear in octopuses (Anderson *et al*., 2002). Individuals were captured over the course of 11 months between February and December 2010. *P. cordiformis* are only spring-summer breeders (Anderson, 1999), and so collection mostly (but not entirely) occurred outside of these seasons. Senescence is therefore unlikely to have heavily affected results. Senescence typically lasts for around a month (Anderson *et al*., 2002), so strong effects on behaviour and physical state (such as skin lesions, retraction of skin from the eyeball and non-directed activity; Anderson et al., 2002) would have been observed if any of the octopuses were senescent during acclimation or trials, none of which were noted. In particular, cessation of appetite is observed during senescence (Anderson *et al*., 2002), which was absent in this study except for one octopus, for whom other indicators of senescence were absent.

Baer (1998) found that increased activity can be an indicator of stress in some animals, although activity was not chosen as a stress indicator for this study due to the positive relationship between enrichment and activity in octopuses (Rehling, 2000, but see Anderson

& Wood 2001). My aquaria (71cm x 100cm x 70cm ; height x length x width) held little enrichment because of the controlled structure of trials, and Anderson & Wood (2001) suggest that the relative influence of enclosure size may vary with octopus size. Due to the large foraging ranges of octopuses in the wild (Forsythe & Hanlon, 1997), it is possible that the size of the housing was unsuitable for long-term captivity. This is an area which, while beyond the scope of this study, merits further exploration. Nevertheless, the lack of variation in exploratory behaviour over time and the low expression of stress-related activities found in this study point to robust behavioural responses in *P. cordiformis* to capture and short-term residence in a captive environment.

Other studies have used sterotypies such as grooming (Balcombe *et al*., 2004) and self-mutilation (Wickens & Heleski, 2010) as proximate measures of stress, which may be more appropriate than food-rejection and activity. Both of these behavioural parameters were used in the present study and, while it is difficult to interpret non-significant results, the lack of differences between animals over time may further suggest an overall lack of stress in the study subjects. The use of limb-damage as a proxy for stress should be considered with caution, as it is unclear whether chronic stress from mutilation is present.

Little is known about the behaviour of *P. cordiformis*, and while many parallels can be drawn from the more commonly studied species of octopuses (*Octopus vulgaris* (e.g. Fiorito *et al.*, 1990; Mather & O'Dor, 1991; Fiorito & Scotto, 1992; Hochner *et al*., 2003; Brown *et al*., 2006; Anderson *et al*., 2008; Gutnick *et al*., 2011)*, O. cyanea* (e.g. Yarnall, 1969; Forsythe & Hanlon, 1997; Mather & Mather, 2004) and *O. bimaculoides* (e.g. Boal *et al*., 2000; Sinn *et al*., 2001; Hvorecny *et al*., 2007)), some of these cannot be extrapolated to distantly related or ecologically segregated species. Rehling (2000) suggested that enrichment, for example, stimulates an increase in octopus activity, yet Anderson & Wood (2001) found that enrichment had no effect on the giant Pacific octopus (*Enteroctopus dofleini*; but see Dickel *et al*., 2000). Habitat size is frequently a problem in animal studies (Hulberg & Oliver, 1980; Ödberg, 1987; Anderson & Wood, 2001; Marashi *et al*., 2003), but it is difficult to discern whether this was the case in this study. A closer examination of *P. cordiformis* acclimation focused on physiological aspects of acclimation and using robust indicators of stress would be advantageous before strong conclusions are drawn from these data. This would, however, require a much more extensive and potentially invasive sampling effort, which is not currently achievable. Instantaneous blood sampling and cannulation are not ideal due to

restrictions on mobility imposed by cannulae and the tendency for octopus stress hormone levels to spike during handling (Malham *et al*., 2002). In the event that acclimation has not occurred within the three-day timeframe that was set in this study, future studies should use a longer acclimation period to examine long-term effects, integrating blood-chemistry assessment as technology permits.

Future studies should make use of the short-term adaptability seen in *P. cordiformis* to consider questions about behaviour of wild-caught octopuses in comparison to lab-reared specimens, and to use these observations for comparison with behaviour from long-term captivity studies. This would allow investigation of whether there is a threshold for how long wild octopuses may be involved in behavioural trials to provide valid data. The results from this study suggest that *P. cordiformis* is a viable choice for short-term behavioural trials.

Chapter 4: Sleep Deprivation

Introduction

Sleep is an important activity among a large portion of the animal kingdom. This behaviour is taxonomically widespread and varies dramatically with ecotype, being found in different forms in aquatic, aerial, arboreal and subterranean environments (Capellini *et al*., 2008; 2009). The majority of animals that exhibit sleep are thought to be vertebrates (Siegel, 2005; Capellini *et al*., 2008), and the vast majority of published material examining sleep and sleep deprivation focuses on higher vertebrates, especially birds and mammals (Zeplin *et al*., 2005; Capellini *et al*., 2008; although see McNamara *et al*. 2009). One of the rare exceptions to this is *Octopus vulgaris*, which was recently discovered to exhibit brain activities that were analogous to slow and fast-wave patterns seen during sleep in higher vertebrates (Brown *et al*., 2006). Octopuses exhibit complex behaviour, such as sophisticated memory and learning, similar to some higher vertebrates (Wells, 1966; Boal, 1991; Fiorito & Scotto, 1992; Boal *et al*., 2000; Alves *et al*., 2007), yet lack a contiguous phylogenetic relationship from which sleep behaviour could arise (Nielsen, 1999). However, the focus of sleep research on vertebrates may potentially mask selective pressures that have influenced the evolution of sleep. The potential parallel evolution of sleep in an intelligent invertebrate (octopus) offers an opportunity to examine sleep outside of the well-studied arena of vertebrates. It also permits exploration of the function and evolution of sleep, a focus which remains both contentious and unresolved (Rechtschaffen, 1998; Siegel, 2005; Stickgold, 2005; Tononi and Cirelli, 2006; Lima and Rattenborg, 2007; Rattenborg *et al*., 2007; Lesku *et al*., 2008).

Defining sleep

Sleep is classically defined through either behavioural criteria or through electrophysiological criteria (see McNamara *et al*., 2009). The behavioural criteria used in determining sleep include species-specific posturing, behavioural dormancy, and a rapid reversibility of state which is not seen in metabolic physiological states such as torpor and coma (Hartse, 1994). An additional characteristic is that the behaviour is under homeostatic regulation (Denlinger *et al*., 2001; Tobler, 2005). Sleep deprivation generates positive behavioural feedback, so that lost sleep is recovered in a process known as ‘sleep rebound’. Sleep rebound is a critical

consideration, as it allows true sleep to be distinguished from the natural circadian activity cycles that are widely observed in non-sleeping animals (McNamara *et al*., 2009).

The second approach used to characterise sleep is through the use of electrophysiological criteria. Two distinctive, cyclical phases of brain activity occur during sleep (McNamara *et al*., 2009). These phases are rapid eye movement (REM) sleep and non-REM (NREM) sleep. REM sleep consists of low-voltage, mixed-frequency measurements from electroencephalograms (EEG), limited body control seen as twitches in extremities, reduced muscle tone, and rapid eye movements (Siegel, 2005). NREM sleep is characterised by high-amplitude cortical EEG slow waves, and is associated with lowered stimulus sensitivity thresholds (McNamara *et al*., 2009). Both REM and NREM states of sleep have been suggested to play roles in different kinds of learning and metabolic regulation and recovery, but the findings from studies on the roles of REM and NREM sleep differ widely, and can vary between species and ecotypes (Capellini *et al*., 2008; 2009). Furthermore, different methodologies can yield variable results regarding the role of sleep in brain development, behaviour, and metabolic processes (Capellini *et al*., 2008; 2009).

The literature examining sleep in invertebrate species is sparse by comparison with that of vertebrates (McNamara *et al*., 2009). Most of the invertebrates examined so far have failed to fulfil all the criteria for 'true sleep' (e.g. cockroaches; Tobler & Neuner-Jehle, 1992; scorpions; Tobler & Stalder, 1988). Although many of the behavioural criteria for sleep have been observed in a few species (e.g. honeybees (Kaiser, 1988; Kaiser, 2002); fruit flies (Hedricks *et al*., 2000; Shaw *et al*., 2000); scorpions (Tobler & Stalder, 1988); cockroaches; Tobler & Neuner-Jehle, 1992)), few studies have used EEG to test the brain activity of invertebrates during 'sleep' (but see McNamara *et al*., 2009 for review). However, findings from both behavioural and EEG studies support the evolution of sleep in invertebrates. The cases for honeybees (*Apis mellifera;* e.g. Kaiser, 1988; Kaiser, 2002) and fruit flies (*Drosophila melanogaster;* e.g. Hedricks *et al*., 2000; Shaw *et al*., 2000) are especially compelling. Both species have been shown to meet the criteria of behavioural quiescence, species-specific posturing and heightened arousal thresholds coupled with rapid state-reversibility and homeostatic regulation (McNamara *et al*., 2009). These are supported by a suite of pharmacological knockout studies which show that this homeostatic regulation is distinct from circadian regulation present in animals that do not fulfil criteria for 'true sleep' (reviewed in McNamara *et al*., 2009).

The prevalence and role of sleep in invertebrates with complex behaviour comparable to that of higher vertebrates has yet to be closely examined. The function of sleep as a regulator of cognitive function is strongly evidenced by many researchers (Hobson, 2005; Siegel, 2005; Capellini *et al*., 2008), but contested by others that suggest sleep evolved primarily for metabolic and maintenance purposes (see McNamara *et al*., 2009). The current study seeks to establish information about 'sleep' patterns in octopuses, which will allow the inclusion of 'intelligent' invertebrates in the ongoing efforts to determine the cognitive and metabolic impacts of sleep, and to unravel the ecological determinants of the evolution of sleep.

Sleep and cephalopods

Cephalopods are recognised as the most cognitive of all invertebrates (Mather, 2008). They have levels of behavioural plasticity (Hanlon *et al*., 1999; Hochner *et al.*, 2006), spatial memory (Boal *et al*., 2000; Alves *et al*., 2007), and associative (Boal, 1991), observational (Fiorito & Scotto, 1992) and tactile learning (Wells, 1966) that rivals those of some vertebrates. Befitting these capacities, cephalopods have complex nervous systems which co-ordinate visuospatial interpretations with motor activity and working memory simultaneously (Mather, 2008). They also have the largest brain/weight ratio of invertebrates (Packard, 1972), exceeding that of reptiles and comparable to some birds and mammals (Young 1971; Packard 1972).

In the only published study to examine sleep deprivation in cephalopods, Brown *et al*. (2006) found both behavioural and EEG evidence for the presence of sleep in *O. vulgaris*. Post-trial observation of octopuses deprived of sleep for 12 hours showed significant homeostatic rebound in 'quiet' states (sleep could not be disentangled from resting states with eyes closed), whereas no rebound was seen in non-deprived subjects. Furthermore, sleep-deprived individuals had greater attack latencies towards a trained stimulus. EEG examination of octopuses in the same study also revealed increases slow-wave brain activity during quiet states (Brown *et al*., 2006), analogous to increases in slow-wave activity in sleeping vertebrates during NREM sleep (Bullock & Başar, 1988). This slow-wave activity and NREM sleep are thought to play a role in episodic memory (Walker & Stickgold 2006; Sterpenich *et al*., 2007; Walker, 2008a; Walker, 2008b). The findings by Brown *et al*. (2006) not only support the evolution of sleep in a cognitively complex invertebrate, but also provide

support for functional parallels in the evolution of sleep between vertebrate and invertebrate species.

Convergence

The common ancestor to vertebrates and cephalopods is thought to predate the phylum Chordata, a simple organism lacking in a complex nervous system (Nielsen, 1999). The prevalence of sleep in animals with complex and cognitive behaviour (e.g. birds and mammals; McNamara *et al*., 2009) has led to the *hypothesis* that sleep contributes to learning and memory consolidation (Siegel, 2001; Vertes, 2004; Walker 2008a; Walker 2008b), and brain development (Marks *et al*. 1995). In octopuses, learning and memory seem to be facilitated by neural circuitry that is functionally similar to that found in higher vertebrates (such as the vertical lobe; Fiorito & Chichery, 1995; Fiorito *et al*. 1998). Rattenborg *et al*. (2009) suggested that "the convergent evolution of homeostatically regulated SWS (slow wave sleep) in mammals and birds was directly linked to the convergent evolution of large, heavily interconnected brains capable of performing complex cognitive processes in each group". The high intelligence of cephalopods, their observational learning abilities (Fiorito & Scotto, 1992) and excellent spatial memory (Alves *et al*., 2007), combined with Brown *et al*.'s (2006) EEG evidence for slow-wave activity during sleep states, support the hypothesis that the evolution of sleep may have facilitated complex learning and memory consolidation in intelligent animals.

Using octopuses as study subjects allows us to test the effects of sleep deprivation on an intelligent, large-brained animal without recourse to ancestry as an explanatory variable. Very little is known about the way in which sleep deprivation impacts octopus behaviour and wellbeing. If sleep does play a part in octopus learning and behaviour, it is important to first establish how sleep deprivation influences the behaviour of octopuses, so that cognitive trials can be conducted upon a solid framework.

Previous comparative studies point towards significant interspecific variation in sleep parameters. These differences arise from functional and/or ecological constraints (Siegel, 2005; Capellini *et al*., 2008). With this in mind, this study measures the behaviour of the littoral marine predator *Pinnoctopus cordiformis* under sleep-deprived and non-deprived conditions to test for the presence of homeostatic regulation of these behaviours. This study

seeks to help establish information about 'sleep' patterns in octopuses, allowing the consideration of intelligent invertebrates in ongoing efforts to determine the cognitive and metabolic impacts of sleep.

Methods

It was important to ensure that the level of alertness (awake versus sleeping) of each octopus could be measured reliably. To facilitate this, rocks constructing the aquarium dens (see Chapter 2) were removed for the period of the trial, and placed around the aquarium in such a way as to prevent subject's eyes from being obscured from view.

Sleep deprivation trials began at 7am on the morning following the completion of acclimation (Chapter 3). Light cycles followed ambient sunlight levels transmitted through the aquarium windows. Octopuses were randomly allocated to one of two treatments: deprived of (-), or allowed (+), sleep , with the octopus in the latter group left undisturbed for 12 hours (Brown *et al*., 2006). Sleep deprived individuals were checked every 10 minutes during the 12 h sleep deprivation period. Animals observed to be 'sleeping' (defined in Chapter 2; eyes closed, not distended, and sometimes covered by folds of skin surrounding the eye) were stimulated to wakefulness first by tapping on the side of the tank, and then by gentle stimulation with a wooden pole. When conducting 'sleep allowed' studies, the aquarium was still entered at 10 min intervals to control for the potential influence of sound and vibration (André *et al*., 2011). Following the 12 h experimental period the animals were left undisturbed and their behaviour was filmed for the subsequent 24 h using the same methods used during acclimation trials (see Chapter 3). Behaviour was then scored from this footage as described in Chapter 3.
Data consisted of count and proportion data, and were analysed and graphed in Prism 5.0a for Mac OS X. Data were non-normal, and were consequently analysed using Kruskal-Wallis tests (for analysis of multiple groups) and Mann-Whitney U tests (for pairwise comparisons). Where multiple pairwise comparisons were necessary, Bonferroni corrections were made.

Results

Activity

Sleep deprivation did not influence the amount of sleep observed in sleep deprived relative to non-deprived *P. cordiformis* (Mann-Whitney U = 37.0, p = 0.829; Fig. 4.1). On average, sleep deprived octopuses slept for 128.25 $\pm$ 3.84 minutes compared to 127.38 $\pm$ 3.00 minutes slept by non-deprived octopuses. Only two statistically significant effects were observed within the suite of activity behaviours measured. Firstly, sleep-deprived octopuses spent more time luring than non-deprived octopuses (Mann-Whitney U = 18.0, p = 0.050; Fig. 4.2a). This difference approached significance between sleep deprived and non-deprived females (Mann-Whitney U = 1.00, p = 0.057; Fig. 4.2c) where deprived females lured more, but was not significant between sleep deprived and non-deprived males (Mann-Whitney U = 12.0, p = 0.910; Fig. 4.2b). There were no differences in luring activity between sleep-deprived and non-deprived octopuses of different weight or damage categories (see table 2.2).

Secondly, sleep-deprived octopuses groomed more than non-deprived octopuses (Kruskal-Wallis H = 18.0, p = 0.050; Fig.3a; see table 2.1). This difference was driven by females (Mann-Whitney U = 0.00, p = 0.03; Fig. 4.3c), but differences in grooming were not significant for males (Mann-Whitney U = 12.0, p = 0.920; Fig. 4.3b). Grooming activity did not differ significantly between octopuses of different weight or damage categories (see table 2.2)

Locomotion

No differences in locomotion were observed between sleep-deprived and non-deprived octopuses (see tables 2.3-2.4).

Appearance

Undamaged (D1) octopuses exhibited bland body patterning less frequently when deprived of sleep (Mann-Whitney U = 0.00, p = 0.04; see table 2.6). However, no differences were observed between deprived and non-deprived moderately damaged (D2) or heavily damaged (D3) octopuses, nor between sleep-deprived vs. non-deprived octopuses overall (main treatment effect; see table 2.5). After multiple comparisons correction, the difference between

deprived and non-deprived D1 octopuses was non-significant (at p = 0.025). No differences were observed in body pattern between sexes or between weight categories (see tables 2.5-2.6).

D1 octopuses also showed lower levels of smooth body texture when deprived of sleep, an effect which approached significance (Mann-Whitney U = 1.00, p = 0.070; see table 2.6). D2 and D3 showed no observable effect on body texture with sleep deprivation. No differences were observed in body texture between sexes, weight categories, or overall (see tables 2.5-2.6).

Colour expression in sleep deprived vs. non-deprived octopuses did not differ between sexes, weight or damage categories or overall (see tables 2.5-2.64).

Supplementary Observations

Although not quantified, or tested statistically, a number of behaviours were observed during sleep which were of particular interest. Octopuses were sometimes seen to move their limbs in a way that appeared to be aimless during sleep, and this was often accompanied by movement of the eyes despite still being covered by folds of skin. On multiple occasions sleeping octopuses were observed to twitch sharply over (30-120 seconds) while changing texture from smooth to mottled and back, and changing colour rapidly between white and dark red. These changes were numerous over this short space of time, and it was not uncommon for twenty colour changes to be observed. Colour changes often occurred at different rates and in 'patches', and both colour changing and twitching were also accompanied by what appeared to be flinching, where the octopus would flatten itself toward the ground, and the skin around both eyes would contract further around the each eye. This suite of behaviours was only observed at the end of sleeping bouts, and consistently ended with the individual waking.

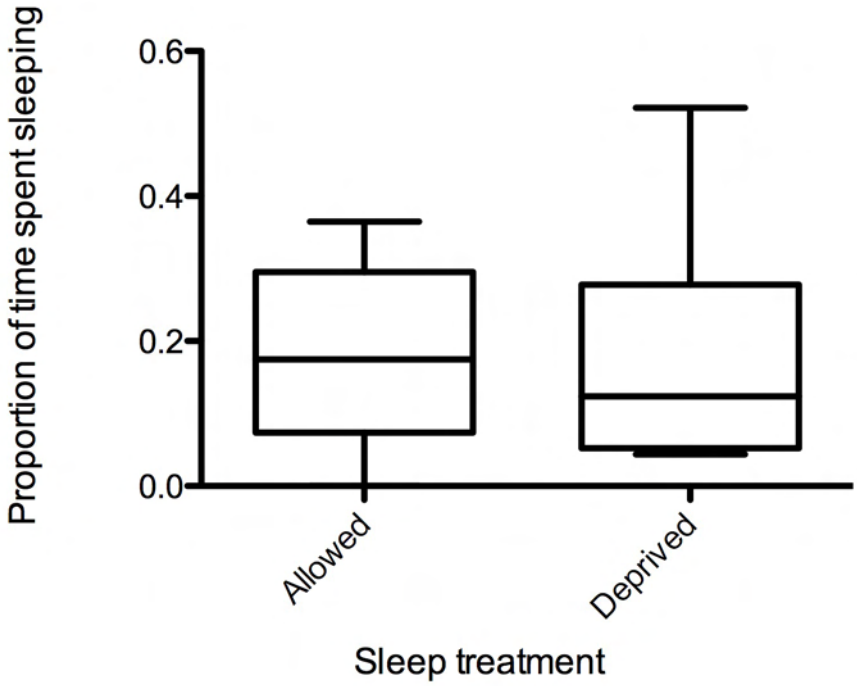

Figure 4.1: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent by *P. cordiformis* sleeping following 12 hours of being allowed (n = 10) or deprived (n = 8) of sleep (Mann-Whitney U test).

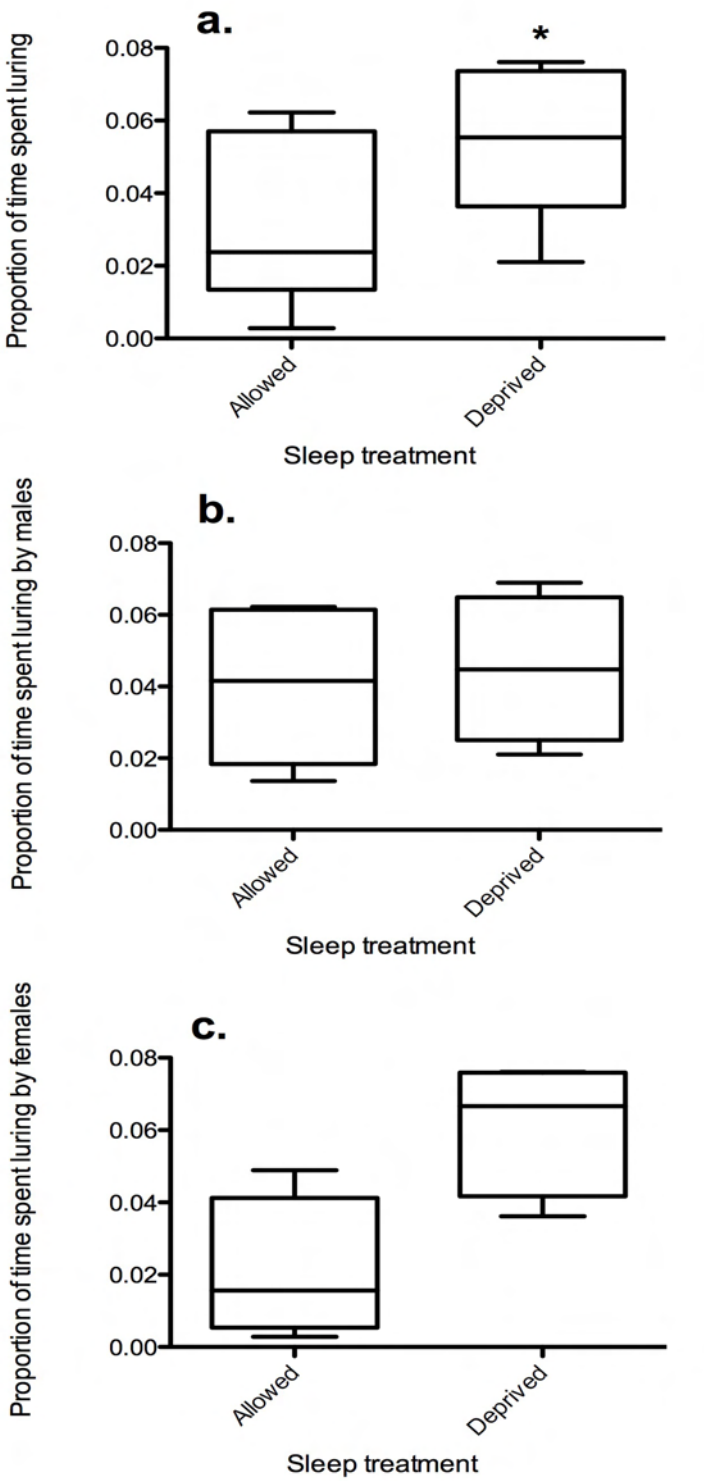

Figure 4.2: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent by *P. cordiformis* luring following 12 hours of being allowed or deprived of sleep; (a) overall (n = 10-8) (b) males (n = 4) (c) females (n = 6-4) (Mann-Whitney U test). * indicates that a treatment differs significantly from the adjacent treatment.

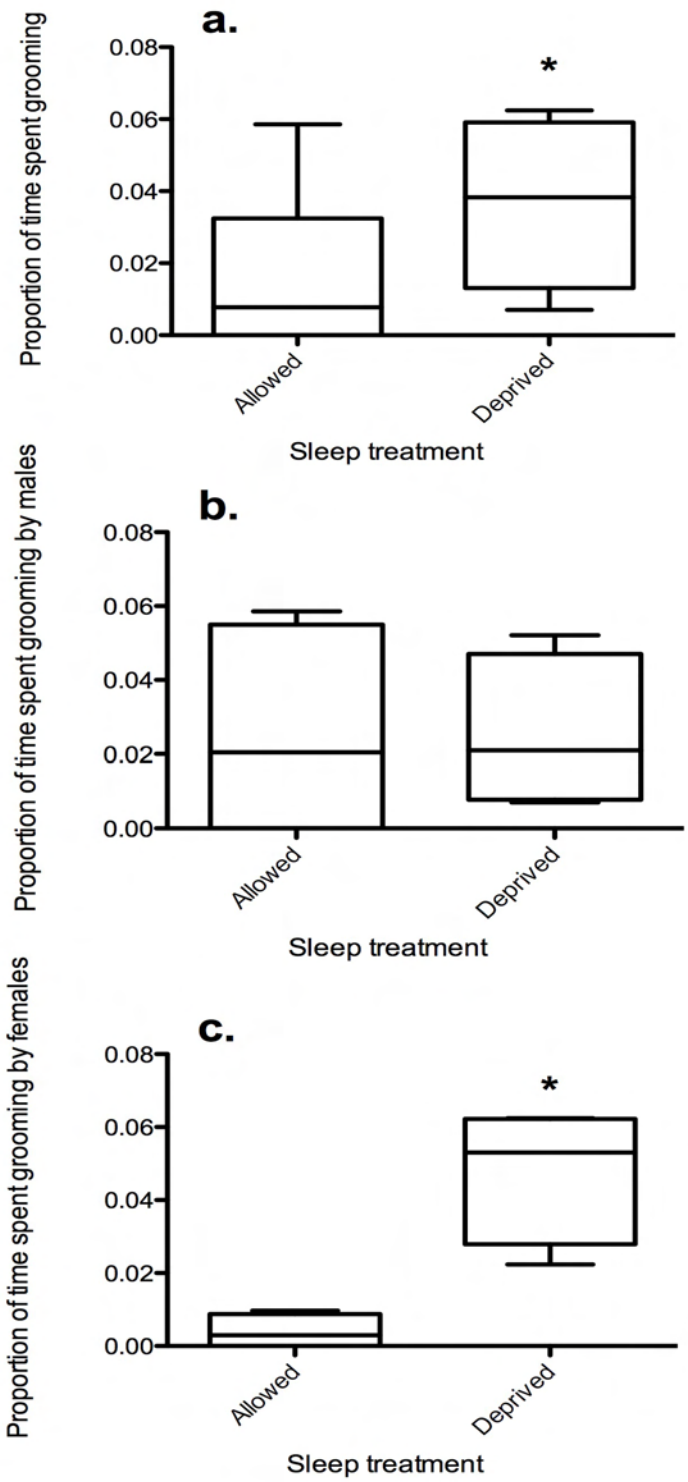

Figure 4.3: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent by *P. cordiformis* grooming following 12 hours of being allowed or deprived of sleep; (a) overall (n = 10-8) (b) males (n = 4) (c) females (n = 6-4) (Mann-Whitney U test). * indicates that a treatment differs significantly from the adjacent treatment.

Discussion

Given evidence for the role of sleep in complex behaviour (see Capellini *et al*., 2009), it might be expected that *P. cordiformis* should exhibit sleep-like behaviour, as demonstrated by *Octopus vulgaris* (Brown *et al*., 2006). In Brown *et al*.'s (2006) study *O. vulgaris* met the common criteria of sleep, with sleep (cautiously referred to as 'quiet behaviour' or 'eyes closed') being homeostatically regulated behavioural quiescence with heightened stimulus thresholds that were separate from circadian rhythms (McNamara *et al*., 2009). The findings of the current study lend some support to observations from Brown *et al*. (2006), and suggest that octopus rest states are analogous to mammalian NREM cycles, although the existence of REM sleep in invertebrates is so far undiscovered (McNamara *et al*., 2009). Siegel (2005) noted that REM sleep is characterised by limited body control, seen as twitches in extremities, rapid eye movement and increased rates of ventilation. Although not tested quantitatively during this study, some acclimating octopuses were observed to move their limbs in a seemingly uncoordinated manner during 'sleep' or quiescence, and in several instances sleeping octopuses were observed moving their eyes underneath the folds of skin that surround the eyeball during sleep. Furthermore, on multiple occasions sleeping octopuses were observed to twitch sharply for a short space of time (30-120 seconds) while changing texture from smooth to mottled and back, and changing colour rapidly between white and dark red. Colour changes often occurred at different rates and in 'patches'. Colour changes and twitching were also accompanied by apparent flinching, with subjects flattening themselves toward the ground, and the skin around both eyes contracting further around the each eye. This suite of behaviours was only observed at the end of 'sleeping bouts, ending with the individual waking. Although not tested statistically, this offers an interesting avenue of investigation for the future. These behaviours could be interpreted as being analogous to the mammalian REM sleep (see McNamara *et al*., 2009), and their consideration in future studies should be fruitful.

In terms of the presence of homeostatic regulation of sleep behaviour in octopuses, results from this study do not strongly conform to results from Brown *et al*. (2006). In that study subjects showed clear behavioural rebounds in alertness levels and modification of attack latencies to a conditioned stimulus following 12-hour sleep deprivation. These parameters were not examined in the current study. Although some differences were seen in the grooming and luring of some subjects (see table 2.1), octopuses in this study did not sleep

significantly more when deprived of sleep for 12 hours. These results suggest that either a 12 hour timeframe was insufficient to elicit sleep rebound in subjects, or sleep is not common to all octopuses.

Sleep deprived *P. cordiformis* slept for 128.25 $\pm$ 3.84 minutes compared to 127.38 $\pm$ 3.00 minutes slept by non-deprived octopuses over the subsequent 24 hours. By comparison, *O. vulgaris* in Brown *et al*'s (2006) study slept or were ('quiet) for approximately 75 minutes over 24 hours before being deprived of sleep, and approximately 285 minutes of 24 hours after being deprived of sleep. Although it seems unlikely that *O. vulgaris* (Brown *et al*. 2006) is subject to sleep rebound and that *P. cordiformis* is not, it is not uncommon for closely related species to respond differently to sleep deprivation.

Of the approximately 50 of the ~60,000 vertebrate species tested for all of the criteria defining sleep, not all of those species meet all of the criteria (Siegel, 2008). In fact, many exhibited greatly reduced or absent sleep states (Siegel, 2008). There is a wide diversity of sleep patterns observed in vertebrates, varying dramatically with ecotype (Capellini *et al*., 2008; 2009) and life stage (McNamara *et al*., 2009). For instance, the amount of time spent sleeping in donkeys amounts to only 3 hours per day, while armadillos sleep 20 hours per day (see Capellini *et al*., 2008). My observations support similar variability in cephalopods, where non-deprived *P. cordiformis* slept for around 2 hours per day, compared to just over one hour in *O. tetricus*.

EEG readings from a number of mammals in marine environments indicate that REM sleep in mammals can be greatly reduced or absent, and that sleep may even occur in only one hemisphere of the brain at a time in some species (Capellini *et al*., 2008). This appears to be driven at least in part by the need to care for offspring, as these reductions are greatest when the development rate of newborns is highest (Siegel, 2005). Predation risk may also be an important factor. Meta-analysis by Capellini *et al*. (2008) showed that sleep quotas in mammals were reduced in sites with higher exposure. This is unlikely to influence den-dwelling octopuses such as *P. cordiformis* (Anderson, 1999) and *O. vulgaris* (Tricarico *et al*., 2011) under normal circumstances, however in novel environments such as aquaria the perceived threats and levels of exposure may differ due to den structure and other environmental enrichment.

The lack of observed sleep rebound in *P. cordiformis* following 12 hours of sleep deprivation may not necessarily be due to differences between the animals used in this study and those

tested by Brown *et al*. (2006). Instead, the methodological approach may have influenced results through observational shortcomings or sampling design. While Brown *et al*. (2006) were better able to accurately verify sleep states with measurements of brain activity, surgical operations on animals, which were not applied in the present study, require an extremely high level of skill to perform, and can potentially confound behaviour.

It is possible that mere observation is not effective as a means of measuring sleep activity. Siegel (2008) noted that "although eye closure is correlated with sleep, individuals can close their eyes for long periods of time with completely unimpaired consciousness". Although their discussion was in relation to mammals specifically, the same logic can be applied here, as throughout residence in the aquaria *P. cordiformis* were observed to spend long periods with their eyes closed or near-closed while remaining relatively unresponsive to external stimuli. This problem is compounded by the lack of recording resolution in the equipment used in this study, which made fine discriminations about whether a subject's eye was completely or only partially closed difficult in some video samples. This problem was also experienced in the study by Brown *et al*. (2006) and may be a limiting factor in their conclusions. This makes it difficult to quantify sleep purely through observation, and more reliable electroencephalogram methods could be a desirable solution to this lack of clarity.

As noted previously, some marine mammals (as well as birds) have been observed to engage in unihemispheric sleep, only sleeping in one half of the brain at a time (Siegel, 2005; McNamara *et al*., 2009). This has been suggested to function as an antipredatory response, as well as allowing animals to migrate (Siegel, 2008; Capellini *et al*., 2008) and tend dependant young for long periods of time (Siegel, 2008; Capellini *et al*., 2008). Throughout residence in the aquaria, octopuses were sometimes observed resting with one eye closed but the other open. This may be an indication that *P. cordiformis* engages in unihemispheric sleep, as seen in cetaceans (Capellini *et al*., 2008). Although such behaviour has not been reported in *O. vulgaris* (Brown *et al*. 2006), it is possible that *P. cordiformis* sleeps unihemispherically to reduce predation risk, or to increase vigilance in the novel captive environment of an experimental aquarium. This is supported by evidence from meta-analysis of mammalian sleep literature showing that mammals adjust their sleep according to perceived threat and exposure (Capellini *et al*., 2008). If octopuses were recorded 'sleeping' with only one eye, the amount of time spent sleeping vs. awake could be under- or over-estimated idiosyncratically depending on the relative subject-to-camera position.

The observation period over which sleep activity was tested may have been too brief for patterns to emerge. In a meta-analysis of studies on sleep characteristics in 127 mammals, Capellini *et al*. (2008) showed that studies which recorded sleep for less than 12 hours underestimated the amount of sleep performed by subjects. In this study a 24 hour timeframe was used to observe post-trial sleep patterns, but only ten minute samples were recorded from each hour. Although sleep in *P. cordiformis* typically lasted for more than 1 hour *(pers obs.)* and so was likely to be observed, it is possible that the length of sleeping bouts was underestimated, although this should be evenly distributed across treatments and it is thus unlikely that the relative amount of sleep differed between groups.

Given the wide range of criteria that can be used to distinguish sleep behaviour from cyclically-regulated behavioural quiescence (McNamara *et al*., 2009), there is a clear need for further metrics to be used in examining sleep behaviour in *P. cordiformis*. Brown *et al*. (2006) noted that sleep-deprived *O. vulgaris* had higher attack latencies in comparison to non-deprived subjects when presented with a conditioned stimulus. Because attack latency was not tested in this study, this parameter is difficult to compare between *P. cordiformis* and *O. vulgaris* sleep behaviour. Stress is often implicated in criticisms of sleep deprivation paradigms (Capellini *et al*., 2009), and it is possible that female octopuses were more strongly stressed by sleep deprivation, stimulating them to groom more frequently in comparison to non-deprived females. Sex-specific responses to sleep deprivation are observed in mammals (e.g. Anderson *et al*., 2008) and may include stress responses (Koehl *et al*., 2006). However, in this study food rejection, used as a proxy for stress, did not rise following sleep deprivation, suggesting that stress levels were not excessive. Alternatively, given the increase in luring behaviour in sleep-deprived individuals (thought to be passive predatory behaviour; Hanlon & Messenger, 1996), it may be that octopuses were trying to recoup lost energy after being deprived of sleep (supported by the lack of food rejection), and groomed more as a response to stress induced by hunger.

Sleep deprivation can be strongly deleterious to some organisms (Rechtschaffen & Bergmann, 2002; Siegel, 2005; Capellini *et al*., 2008), and the results of this study cannot exclude negative effects of deprivation, although the distribution of such effects among species appears somewhat idiosyncratic (McNamara *et al*. 2009). Sleep deprivation of rats can elicit strong metabolic and thermoregulatory changes that lead to death (Rechtschaffen &

Bergmann, 2002). The same sleep deprivation techniques applied to pigeons produce none of these symptoms (Newman *et al*., 2008), and only stimulate some of these symptoms in cockroaches (Tobler & Neuner-Jehle, 1992). Without hormonal and metabolic measurements, the physiological impacts of sleep deprivation in *P. cordiformis* cannot be determined.

Conclusion and future directions.

My results do not agree with those of Brown *et al*. (2006). Although female *P. cordiformis* showed significantly greater grooming behaviour and near-significant luring behaviour post-deprivation, sleep activity was unaffected. This may indicate that environmental pressures, such as predation, have altered *P. cordiformis* responses to sleep deprivation, supported by apparent unihemispheric sleep or rest in my subjects, although further work needs to be done to firmly establish this. The need for further metrics by which sleep deprivation effects could be tested is clear, and examining the influence of sleep deprivation on parameters such as heart-rate, ventilation, blood-hormone levels (Siegel 2008), as well as the use of EEG (Brown *et al*., 2006) would provide a clearer indication of the potential influence (or lack) of sleep deprivation in *P. cordiformis*.

Brown *et al*. (2006) have presented evidence for slow-wave brain activity analogous to that seen in avian and mammalian NREM sleep, and also showed that sleep deprivation induces rebound behaviour in *O. vulgaris*. It remains to be seen whether it also induces changes in brain activity. An examination of the effect of sleep deprivation on slow-wave expression would certainly strengthen the analogy between invertebrate and vertebrate models of sleep structure. Furthermore, REM in many developing mammals is expressed at much higher levels than in adults, which supports suggestions by some researchers that sleep state is important in brain development (Siegel, 2005). Examining the expression of low-voltage REM vs. high-amplitude slow wave NREM activity in octopuses across age gradients would be a viable method of testing the convergence of octopus brain and sleep structure on those of higher vertebrates.

The need to investigate sleep in animals that are phylogenetically distinct is important, and of particular need are comparative studies using invertebrates. Invertebrates typically lack the high levels of intelligence observed in higher vertebrates, but cephalopods offer a unique

opportunity in this sense (Mather, 2008). By continuing investigation of the effects of sleep deprivation on cephalopod brains, researchers may be able to reveal commonalities in the need of highly co-ordinated neural structures to engage in restorative processes, and offer further insight into the selective pressures driving the parallel evolution of sleep among intelligent life.

Chapter 5: Video playback – Response to prey

Introduction

Interpreting cues from the environment plays an important role in the success of nearly all organisms, whether aquatic or terrestrial (Dall *et al*., 2005). The discrimination of specific information allows organisms to adjust their behaviour with respect to predation threat, and opportunities in the form of conspecifics (Lima & Dill, 1990; Ferrari & Chivers, 2011) or food (Dall *et al*., 2005). Information can be communicated between organisms via intentional signals (e.g. Smith & Evans, 2008), but information such as prey identity and location can also be detected and interpreted by unintended receivers (i.e. predators). 'Eavesdropping' on chemical cues (Tomba *et al*., 2001) and acoustic signatures (Lind *et al*., 2005) from prey items allows predators to adjust their behaviour in ways that are unintended by the sender. Furthermore, the way in which predators respond to cues can provide an indication about the sensory modality used by predators when foraging (Cooper, Jr 1994; 1995).

The experimental study of signalling in animals dates back 60 years; some of the earliest attempts to study visual signals in a controlled environment include the use of simplified dummies to present manipulated signals to blackbirds (Tinbergen & Perdeck, 1951). An important drawback to this technique is that it is unable to capture the diversity of sensory information that may be being conveyed (Galoch & Bischof, 2007). It can thus be difficult to standardise individual stimuli in a way that disentangles them from other signals (e.g. isolating acoustic and visual modalities, or in a visual domain, isolating motion cues from cues pertaining to shape). Following from the adoption of audio playback techniques, the advent of video playback in the 1960's has allowed studies to isolate and manipulate specific visual cues with considerable detail (Oliveira *et al*., 2000; Bird & Emery, 2008). Video playback improved visual techniques previously used, where static images were presented to subjects, often resulting in a failure to elicit realistic responses (Ryan & Lea, 1994). Video playback has also allowed experimenters to examine the role of vision in the context of multimodal signalling, and to examine the relative influence of different modes of signalling that occur simultaneously (e.g. Hansknecht & Burghardt, 2010; Smith & Evans, 2011). The development of computer-based techniques to generate models and manipulate parameters has further expanded this growing technique in behavioural research (Butkowski *et al*., 2011), and today video playback manipulations have been used to examine signalling and other

behaviours in a range of animals, including birds (e.g. Rieucau & Giraldeau, 2009; Smith & Evans, 2011), reptiles (e.g. Nelson *et al*. 2010), fish (e.g. Johnson & Basolo, 2003) apes (e.g. Capitanio, 2002), spiders (e.g. Pruden & Uetz, 2004) and recently, octopuses (Pronk *et al*., 2010).

Octopuses are known to have excellent eyesight, but to date little is known about how they use this sensory modality (Mather, 1991; Byrne *et al*., 2002). In 2010, Pronk and colleagues demonstrated for the first time that video playback elicited realistic responses in wild-caught gloomy octopuses (*Octopus tetricus*), and utilised this methodology to demonstrate episodic 'personality' (i.e. correlated suites of behaviour that are temporally discontinuous; Carere *et al*., 2005; Kurvers *et al*., 2009). *O. tetricus* behaviour was examined in response to different objects: a food item, a novel object, a conspecific and a water-filled aquarium control, allowing the researchers to examine behavioural responses of octopuses across a range of different contexts (Pronk *et al*., 2010). However, video playback also offers an opportunity to investigate questions about potential cues that can be used to mediate predatory responses. Here, I use video playback techniques to separately examine the roles of prey motion and prey shape in eliciting predatory behaviour in *P. cordiformis*. Specifically, I test whether altering the appearance and motion characteristics of crabs influences ventilation, appearance characteristics, states of wakefulness, as well as luring and peering behaviour.

Methodology

Procedures

Prior to testing, octopuses were captured and housed using the procedures discussed in Chapter 2, and acclimated to captivity through the procedures discussed in Chapter 3. Following acclimation, octopuses were presented with a series of video clips of a prey item (*Cancer novaezelandiae*). Videos were displayed to octopuses on a Samsung SyncMaster 2243 liquid crystal display LCD computer screen. LCD screens update their pixels instead of flickering, thus minimising problems with flicker fusion rates (Oliviera *et al*., 2000). To reduce external distractions, aquaria were covered on all sides by black polythene.

A 30 minute acclimation period preceded trials. Here, the LCD screen was placed against one side of the tank and baseline footage of acclimation to the display screen was recorded for 30

minutes. This was followed by testing with crab prey stimuli that differed in shape and movement characteristics. Each octopus was presented with each stimulus in random order and responses of octopuses were filmed for behavioural analysis. Responses to each stimulus were scored beginning when a stimulus first appeared on the screen and ended as soon as the stimulus left the screen for the final time in each clip. Continuous behaviours were binned into per-minute averages and then converted into a proportion, and events were examined as per-minute counts.

Stimuli

Playback videos of crabs were obtained from 4 individual crabs to reduce pseudoreplication. Each crab was filmed for 60 minutes in an aquarium using a high definition (1280x720 pixels) Sony DCR-SR67 Handycam. Each crab was then euthanised by mechanical severing of the dorsal ganglion (University of Canterbury ethics permission number: 2009/10R). Following euthanisation, the chelipeds were removed at the base of the limb, and the crabs were fitted with a nylon harness so that motion could be manipulated. Each of these crabs was then filmed making eight transects across the field of view of the recording equipment, with the animal on the proximal side of the aquarium with respect to the recording device. All remaining limbs were then removed from each animal, and a further eight passes of the limbless crabs were recorded. Eight transects were also selected from the 60 minutes of recorded footage of live crabs, where crabs walked along the proximal window of the aquarium.

From these eight transects made by each shape of crab (whole, pincerless and limbless), four for each individual were selected based on the quality of the image and the realism of the motion (e.g. limbless individuals had an unrealistic tendency to float off the ground). The four selected transects were then joined together using Final Cut Express to create the illusion of a crab passing back and forth across the screen with three second intervals between each pass. Each pass lasted between eight and nine seconds. After creating each of these videos, a motionless 50 second image of each individual and each shape was created. In addition, a 3 minute inter-trial video was constructed from footage of the water-filled aquarium, which was used as a break between stimulus clips. This footage was also used to create a 30 minute pre-trial sequence by looping the inter-trial video clip and a reversed version of the inter-trial clip together, and duplicating this loop for 30 minutes.

Analysis

The behaviours that were scored during these tests were ventilation rate, three appearance characteristics (colour, pattern and texture), alertness, luring and peering (see Chapter 2 for definitions).

Ventilation rates were determined by scoring each time an animal breathed. Ventilation was defined as one cycle of expansion and contraction of the mantle. When ventilation could not be measured due to obstruction or rapid movement of the test subject, this was scored as 'out of sight'. To allow for comparison between ventilation rates of animals where ventilation was obscured, ventilation rates were extrapolated by first deducting the amount of time in which ventilation was unobservable before converting breath counts into a per-second measurement. Ventilation counts were recalculated as per-second rates.

Data consisted of count and proportion data, and were analysed and graphed in Prism 5.0a for Mac OS X. Being non-normally distributed, these data were analysed using Kruskal-Wallis tests and Mann-Whitney U tests. Where multiple comparisons were necessary, Bonferroni corrections were made to compensate for the inflation of Type I errors.

Results

Activity

Luring:

P. cordiformis lured significantly more to pincerless (U = 233, p = 0.052) and limbless (U = 210, p = 0.013) crabs compared with the control (Fig. 5.1a; see table 3.1). Female octopuses lured more in response to pincerless (U = 43.0, p = 0.046) and limbless (U = 18.0, p = 0.019) crab playbacks than in response to controls (Fig. 5.1b; see table 3.1), but these differences were not observed in males (see table 3.1). Medium-weight (W2) octopuses lured less in response to pincerless (U = 35.5, p = 0.017) and limbless (U = 23.0, 0.003) playbacks than control videos (Fig. 5.1c), but weight had no other significant influence on luring activity. Similarly, moderately damaged (D2) octopuses lured less in response to pincerless (U = 23.5, p = 0.040) and limbless (U = 6.00, p = 0.002) playback than in response to control videos (Fig. 5.1d; see table 3.1), but no differences were observed for D1 (undamaged) or D3 (heavily damaged) octopuses (see table 3.2).

More luring was observed in response to moving whole (U = 491, p = 0.009) and limbless crabs (U = 528, p = 0.034) compared to motionless homologues (see table 3.3), but there were no differences in luring activity between moving and motionless pincerless stimuli (Fig 5.2).

Alertness:

There were no significant differences between alertness levels in response to different prey shapes (Fig. 5.3a; see table 3.1) or motion (see table 3.3). However, when delineated by sex, female octopuses showed greater alertness in response to limbless prey playback in comparison to whole prey (U = 34.5, p = 0.007), pincerless prey (U = 59.0, p = 0.020), and in comparison to control videos (U = 16.5, p = 0.001; Fig. 5.3b).

Weight and damage also played a small role in mediating alertness levels in response to video playback. Compared to controls, W2 *P. cordiformis* were more alert in response to whole (U = 28.0, p = 0.030) and limbless (U = 24.0, p = 0.006) prey playback (Fig. 5.3c), although no other differences were observed for different weight categories (see table 3.4) D2 octopuses were more alert in response to whole prey playback than in response to control videos (U =

1.00, p = 0.001; Fig. 5.4a), and D3 octopuses were more alert in response to limbless prey than to pincerless prey playback (U = 12.5, p = 0.016; Fig. 5.4b).

Appearance

Prey shape had no effect on any appearance characteristics (colour, pattern and texture) of octopuses (see table 3.5). However, *P. cordiformis* showed a reduced expression of baseline colour in response to moving pincerless prey playback compared to motionless playback (U = 476, p = 0.030; Fig 5.5a). *P. cordiformis* also reduced expression of bland body pattern in response to moving whole prey playback in comparison to motionless whole prey playback (U = 476, p = 0.030; Fig. 5.5b). Finally, video playback only stimulated one significant difference in body texture in *P. cordiformis*, with octopuses having greater expression of smooth texture in response to moving limbless playback compared to motionless limbless playback (U = 288, p < 0.001; Fig. 5.5c; see table 3.7). No significant effect of sex, weight damage were observed in response to shape (see tables 3.5-3.6) or motion (see tables 3.7-3.8)

Reactivity

In comparison to the equivalent moving playbacks, octopuses peered less in response to motionless playback of whole (U = 463, p = 0.001), pincerless (U = 531, p = 0.042), and limbless (U = 504, p = 0.013) crabs than in response to moving homologues (Fig. 5.6, see table 3.9). No other influences of prey shape or movement were observed (tables 3.9 & 3.11), nor were differences found between octopuses with differing sexes, weights or levels of damage (see tables 3.9-3.12).

Prey shape had an effect on ventilation rate, with octopuses reducing ventilation in response to whole prey playback in comparison to limbless (U = 265, p = 0.001) and control (U = 163, p = 0.032; Fig. 5.7a) tests. Octopus ventilation rates also approached significant reductions in response to pincerless prey playback compared to limbless playback (U = 373, p = 0.061) but not to control playback (U = 176, p = 0.082; Fig. 5.7a). Female *P. cordiformis* showed lower ventilation rates in response to whole prey playback compared to limbless playback (U = 27.0, p = 0.007), but otherwise no differences were observed for either sex (Fig. 5.7b; see table 3.11). Lightweight (W1) octopuses had lower ventilation rates in response to whole prey playback compared to control videos (U = 11.0, p = 0.050), and in response to pincerless playback compared to control videos (U = 9.00, p = 0.041; Fig. 5.7c). Heavily damaged (D3)

octopuses ventilation rates approached significantly higher in response to whole prey videos than control videos (U = 8/00, p = 0.059).

Ventilation rates were also lower in response to moving whole prey compared to responses to motionless whole prey (U = 388, p = 0.044), but higher in response to moving limbless prey compared to motionless limbless prey (U = 281, p = 0.005; Fig. 5.8). No differences were observed between sexes, weight or damage categories in response to moving and motionless prey playback (see tables 3.11-3.12).

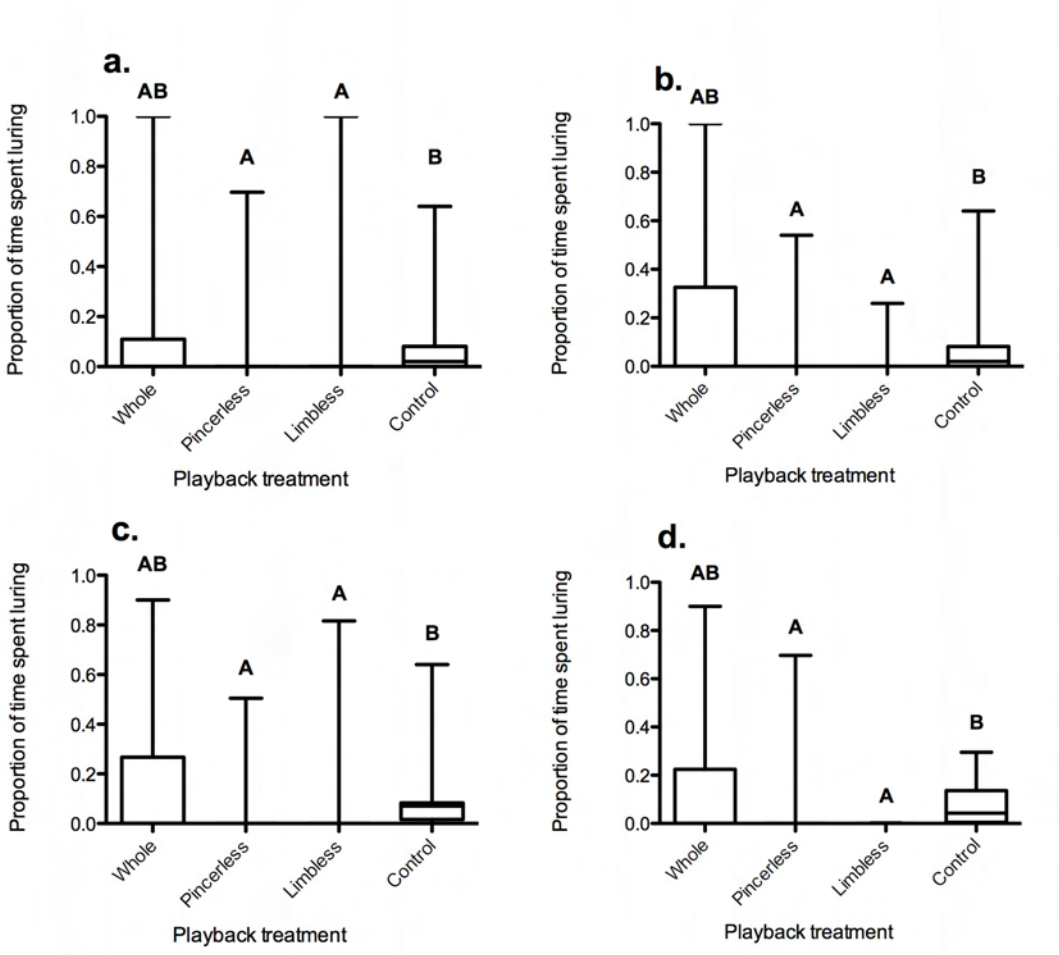

Figure 5.1: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent luring by *P. cordiformis* in response to video playback of a whole, pincerless, or limbless prey item and control (a) overall (n = 19) (b) females (n = 8) (c) medium weight (W2; n = 9) (d) heavily damaged (D3; n = 6) (Mann-Whitney U test). Plotted points sharing letters are not significantly different.

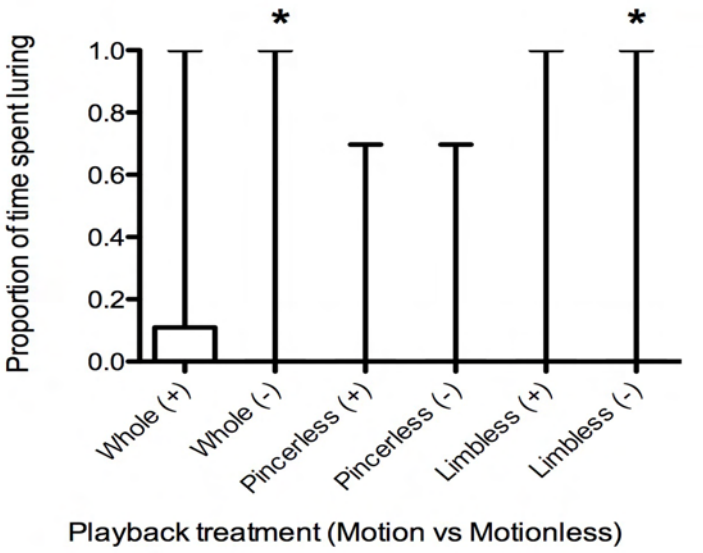

Figure 5.2: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent luring by *P. cordiformis* in response to moving (+) and motionless (-) video playback of whole, pincerless, or limbless prey items (n = 19) (Mann-Whitney U test). Stars denote significant differences relative to moving homologues of prey.

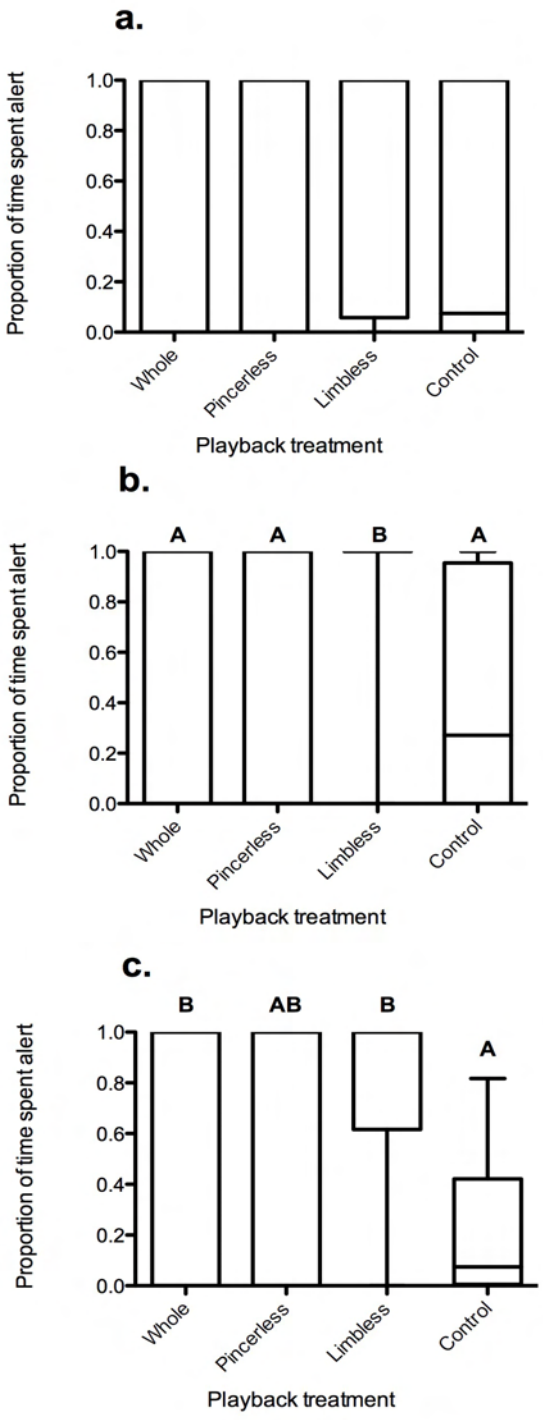

Figure 5.3: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent alert by *P. cordiformis* in response to video playback of whole, pincerless, or limbless prey items and negative controls (a) overall (n = 19) (b) females (n = 8), (c) medium weight (W2; n = 9) (Mann-Whitney U test). Although points to not appear to differ, differences are significant when arranged by rank in non-parametric analyses. Plotted points sharing letters are not significantly different.

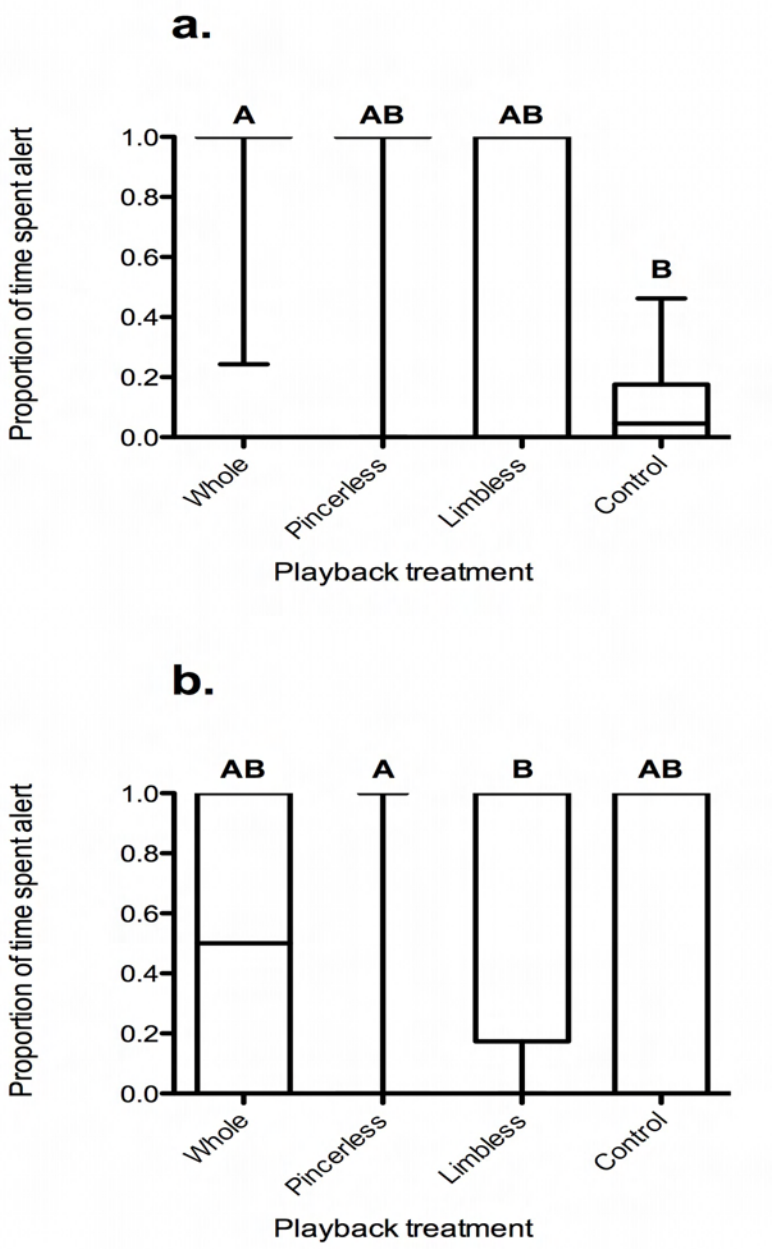

Figure 5.4: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent alert by *P. cordiformis* in response to video playback of whole, pincerless, or limbless prey items and negative controls (a) moderately damaged (D2; n = 6) (b) heavily damaged (n = 6) (Mann-Whitney U test). Although points to not appear to differ, differences are significant when arranged by rank in non-parametric analyses. Plotted points sharing letters are not significantly different.

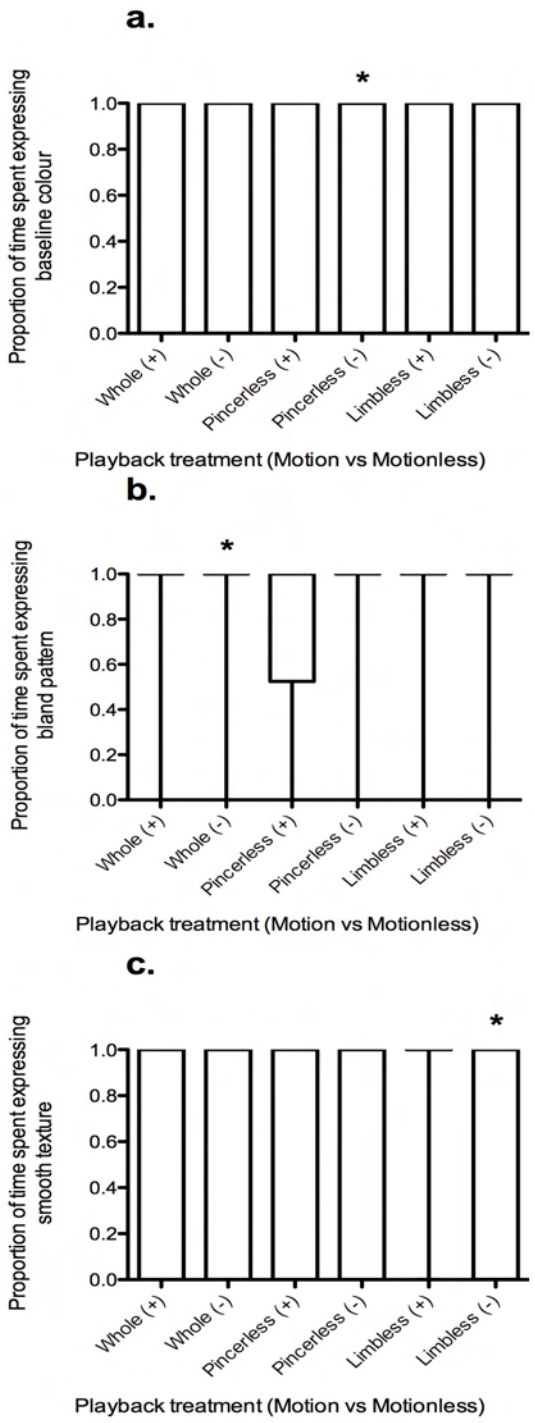

Figure 5.5: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent luring by *P. cordiformis* in response to moving (+) and motionless (-) video playback of whole, pincerless, or limbless prey items (n = 19) (a) colour (b) pattern (c) texture (Mann-Whitney U test). Although points to not appear to differ, differences are significant when arranged by rank in non-parametric analyses.

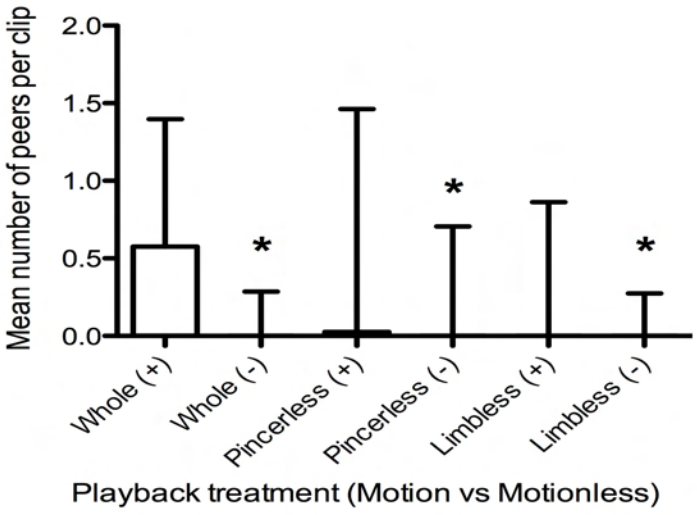

Figure 5.6: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the mean number of peers by *P. cordiformis* in response to moving (+) and motionless (-) video playback of whole, pincerless, or limbless prey items (n = 19) (Mann-Whitney U test).

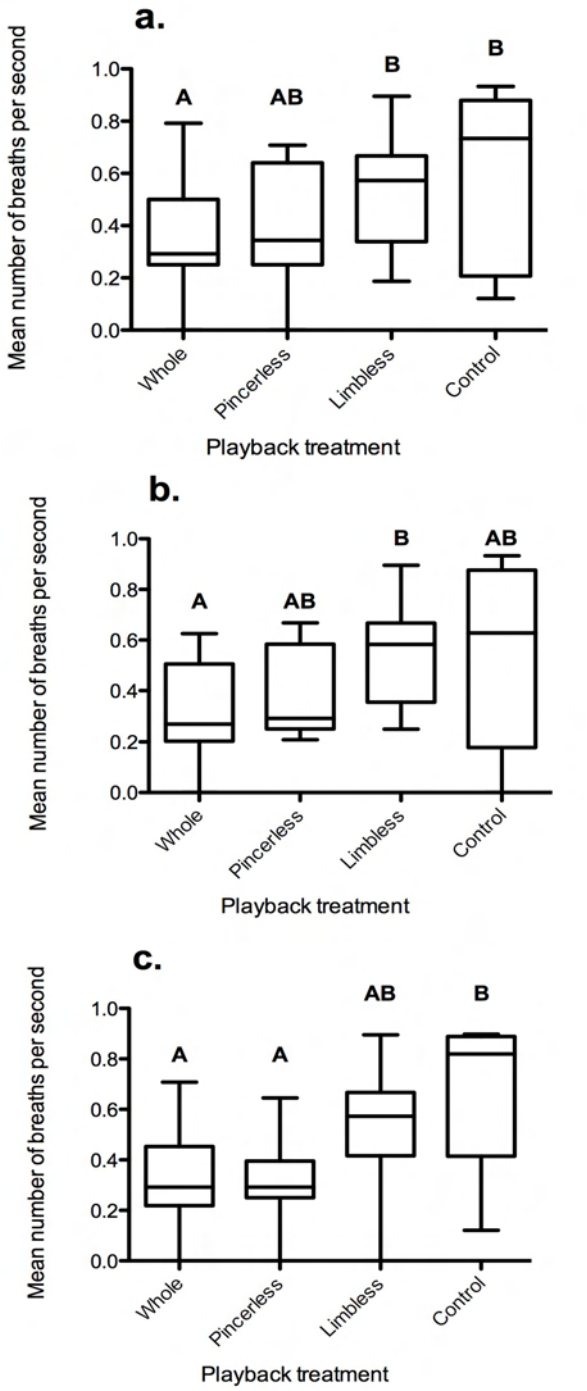

Figure 5.7: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the mean number of breaths per second by *P. cordiformis* in response to video playback of whole, pincerless, or limbless prey items and negative controls (a) overall (n = 19) (b) females (n = 8) (c) lightweight (W1; n = 5) (Mann-Whitney U test). Plotted points sharing letters are not significantly different.

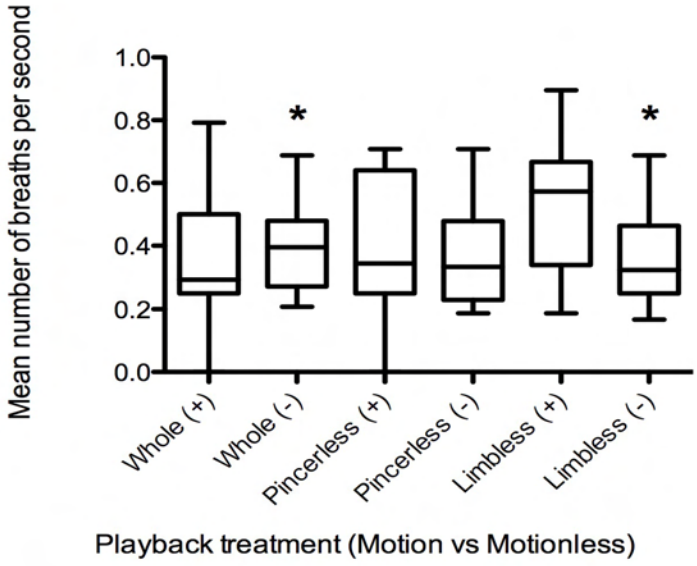

Figure 5.8: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the mean number of breaths per second by *P. cordiformis* in response to moving (+) and motionless (-) video playback of whole, pincerless, or limbless prey items (n = 19) (Mann-Whitney U test).

Discussion

Does P. cordiformis respond to video playback?

Results from this study indicate that the predatory responses of *P. cordiformis* are affected by both prey shape and motion. Of these two parameters, motion appeared to be more important at eliciting a predatory response than shape. Of the behaviours tested, it appears that the strongest response to prey video playback is seen in the way that octopuses adjust their rates of ventilation, which differed depending on both shape and motion, as well as the sex, weight and limb damage of each individual. These results support work by Pronk *et al*. (2010) and show, for a second octopus species, that video playback can invoke context-relevant responses in octopuses.

More realistic prey shape tended to raise the level of alertness observed in *P. cordiformis,* and stimulated higher luring in response to pincerless and limbless crabs than luring observed in response to control playbacks. Although octopuses are classically known as active chemotactic foragers (Chase & Wells, 1986), these results suggest that *P. cordiformis* may also engage in passive, sit-and-wait foraging techniques, luring prey with the tip of an appendage or appendages. Support for this idea comes from the fact that octopuses also adjusted their rates of ventilation while viewing different shapes. By holding their breath and contracting the mantle, cephalopods can create positive pressure inside the mantle, allowing them to expel water through the animals' siphon, jetting a short distance at high speed (Sinn, *et al*., 2001; Huffard, 2006). Although this is commonly used as an escape mechanism, it also has application in foraging contexts by allowing individuals to close a short gap at high speed to capture prey (Forsythe & Hanlon, 1997). Whole prey item videos stimulated lower ventilation rates compared to limbless prey and control playback, suggesting that *P. cordiformis* may have been preparing for jetting, and that undamaged or naturally shaped prey items might generate stronger motivation for the individual to forage.

Just as more realistic prey shapes stimulated lower rates of ventilation, possibly in preparation for jetting and pouncing, moving prey stimulated reductions in ventilation rate in octopuses viewing moving whole and limbless prey compared to the motionless homologue. The idea that undamaged or naturally shaped prey generate stronger foraging motivation is also supported by higher levels of alertness in response to videos of more 'complete' prey in this

study, although it should be noted that these responses were only observed with respect to specific traits (e.g. females responded but no significant differences were observed in males; D2 individuals responded to shape while D1 and D3 individuals did not). There are a number of reasons that such patterns might emerge, which are discussed in more detail below. It is worthwhile first to note that similar patterns were observed in response to motion vs. motionless prey item playbacks.

Interestingly, *P. cordiformis* lured more in response to motionless whole and limbless crabs than in response to moving videos with the same shape. However, octopuses peered significantly more in response to all three moving playback clips, compared to motionless videos with the same shape, indicating that *P. cordiformis* attend to motion cues. Furthermore, motion stimulated changes in all appearance parameters (colour, body pattern and texture), while shape had no effect on appearance, suggesting that octopuses adjust their appearance in response to prey motion.

Differences were also observed in the responses of octopuses of different sex, weight and damage categories, and although seemingly disparate, these results are also revealing. Direct comparisons between male and female *P. cordiformis* were non-significant. However female octopuses did alter their expression of luring, alertness and ventilation rates in response to prey shape; patterns which were not observed among male *P. cordiformis*. While these results may be an artefact of methodological confounds or low sample sizes, these effects suggest that shape may influence female *P. cordiformis* more strongly than it does males, highlighting the need to consider sex in behavioural analyses. There were also differences in behaviour based on octopus weight, although patterns are difficult to discern. While the causes for these changes are unclear, both body weight and the condition of individual *P. cordiformis* do appear to play roles in mediating their responses to video playback, opening an avenue for further examination in the future.

Active forager or passive predator?

This study shows that vision may play an important role in the predatory behaviour of *P. cordiformis*. This may be somewhat surprising, as the marine environment around Kaikoura is subject to high turbidity, often being obscured by transient particulate matter, often reducing visibility to less than 50 cm (Reid *et al*., 2011; pers. obs.). Furthermore, octopuses are known

to be speculative chemotactic foragers, executing pounces to capture prey from likely microhabitats, thus not relying heavily on vision for foraging (Hanlon & Messenger, 1996; Forsythe & Hanlon, 1997). *P. cordiformis* is distributed widely between the Chatham Islands (Anderson, 1999) and southern Australia (Norman & Reid, 2000), and so persists in waters where turbidity would not inhibit visual predatory behaviour. Cooper Jr (1994; 1995) suggests that reliance on chemical cues is an indication of a highly active predator where, by comparison, reliance on visual cues is typified by passively foraging predators (Cooper Jr, 1995). However, it is evident that visual cues are also used by active predators (e.g. Bauer, *et al*., 1977; Neuhofer *et al*., 2009). Balent & Andreadis (1998) made the point that predators generally lie along a continuum between sedentary and active foragers. Thus reliance on visual and chemotactic (as well as other cues) need not be mutually exclusive. Evidence from this study supports the hypothesis that *P. cordiformis* engages in visually-based foraging, as demonstrated by the influence of shape and motion on behaviours such as ventilation rates and luring, where these visual cues derived from the video playback have stimulated context-specific responses.

Although this study has not formally examined the role of chemical cues in mediating foraging behaviour in *P. cordiformis*, preliminary work suggested that adding water in which prey items had been stored may raise the overall alertness of octopuses during trials, with concurrent reductions in ventilation rates (unpublished observation). These observations suggest that robust examination of the influence of chemical cues might offer an avenue of inspection for future studies. Previous studies have shown that other cephalopod species perform better in cognitive trials in the presence of chemical cues (Anderson & Mather, 2010). Furthermore, a number of studies on other taxa (e.g. Galoch & Bischof, 2007; Campbell & Hauber, 2009; Partan *et al*., 2009) have suggested that the presentation of multiple modes of sensory cues may further enhance responses; a phenomenon known as "sense complementarity" (see Hasson, 1989; Møller & Pomianowski, 1993; Partan, 2004; Partan & Marler, 1999; 2005 for discussion). Given that octopuses use speculative chemotactile foraging (Hanlon & Messenger, 1996; Forsythe & Hanlon, 1997) and hunt a diverse range of mobile prey (Anderson *et al*., 2008), foraging efforts would benefit from the integration of multiple sensory inputs.

Mechanics and caveats

This is the first study that investigates specific aspects of visual stimuli in coordinating octopus responses with respect to foraging behaviour. Video images can generate realistic responses in a wide range of taxa (Oliveira *et al*., 2010), but methodological refinement is ongoing. While these results provide cause for optimism, it is prudent to be cautious. Due to the disparity between the visual systems of humans and other animals, many of the video recording and playback apparatuses being employed (including those used in this study) do not necessarily record/display visual information in the exact way that real images would be interpreted by the visual system of the animal being examined; this interpretation may vary not only between apparatuses, but also between species (Fleishman *et al*., 1998). For example, shadows, specular reflections, brightness, luminance and contrast can all influence perception (Zeil, 2000; Rosenthal, 2000; Oliveira *et al*., 2000) and such conditions should approach natural lighting conditions as closely as possible (Endler, 1993; Oliveira *et al*., 2000). Some of the other key considerations for experimenters using video playback to test non-human species include flicker fusion rate, pseudoreplication, and a suite of visual cues including depth perception, motion parallax, brightness, illumination, contrast, spatial resolution, and colour perception (D'Eath, 1998; Cuthill *et al*., 2000; Fleishman & Endler, 2000; Oliveira *et al*., 2000; Zeil, 2000; Riecau & Giraldeau, 2009) as well as non-visual cues such as vibration (Mooney *et al*., 2010) and sound (André *et al*., 2011). Ongoing research has led to the mitigation of a number of the issues raised above, and as Oliveira *et al*. (2000) pointed out, "when used appropriately, video playback allows an unprecedented range of questions in visual communication [or behaviour] to be addressed" (author brackets).

Conclusion

Motion and shape manipulations elicit context-relevant behavioural responses in *P. cordiformis*, supporting the viability of video playback techniques in octopuses. *P. cordiformis* adjusted its behaviour in response to visual cues, indicating that the speculative chemotactic foraging that octopuses engage in (Hanlon & Messenger, 1996; Forsythe & Hanlon, 1997) is either facilitated by, or occurs in addition to, strong attendance to visual cues.

Methodological constraints on video playback can create behavioural artefacts and confound the collection and interpretation of results. However, most of the potential limitations of video playback, as noted by Oliveira *et al*. (2000), are not unique to playback presentation, but relate to most techniques of examining visually-based behaviour. Despite a potentially confounding lack of realistic polarised light cues and the absence of depth cues in the present study, these results reflect similar results obtained by Pronk *et al*. (2010) testing a different species of octopus in the face of identical methodological constraints. It appears clear that cephalopods can be added to the growing list of taxa where video playback technology can be used to successfully examine animal behaviour.

In future, the examination of additional sensory modalities would allow researchers to establish a hierarchy of sensory inputs. For instance, sound vibrations have been shown to result in acoustic trauma in cephalopods (André *et al*., 2011), and may influence the way in which octopuses respond to video playback. As video playback can elicit responses from octopuses, this technique offers great potential for testing other classically examined paradigms, such as mate selection (e.g. Clark & Uetz, 1993; Rosenthal *et al*., 1996; Witte & Klink, 2005), intraspecific aggression (Rowland, *et al*., 1995; McKinnon & McPhail, 1996), communication (Hanlon & Messenger, 1996; Messenger, 2001) and learning responses (Boal *et al*., 2000; Mather, 2008) in octopuses. Furthermore, neural imaging techniques such as those employed by Brown *et al*. (2006) could be utilised in conjunction with video playback presentation to examine octopus brain activity in response to controlled stimuli. This would allow experimenters to present and manipulate stimulus parameters and effectively map response activity to specific regions of the brain, further uncovering the differences and parallels in the structure and function of cephalopod brains with those of vertebrates.

Chapter 6: Video playback – Response to predators

Processing information is critical in the moment-to-moment lives of animals, as correct assessment of environmental information can lead to optimal decisions regarding foraging behaviour, conspecific interactions, and risk assessment (Lima & Dill, 1990; Dall *et al*., 2005; Ferrari & Chivers, 2011). This can confer fitness advantages over individuals with inferior information processing (Helman, 1989; Dall *et al*., 2005; Lönnstedt & McCormick, 2011; Tvardìkova & Fuchs, 2011); while misinterpreting cues about predation and risk assessment can lead to mortality (Abrams, 2000). Because of the high costs associated with failing to identify risk, animals are expected to overestimate the risk of predation (Kavaliers & Choleris, 2001), and to hold some level of vigilance at all times (Montgomerie & Weatherhead, 1988; Lima & Dill, 1990).

Animals often work with incomplete data when cues from the environment are limited (Sih, 1992; Dall *et al*., 2005), expensive to obtain, or beyond the perceptual sampling capabilities of the species in question (Bouskila & Blumstein, 1992). Instead of acquiring full details to inform every decision, it can be more efficient to base decisions on only one or a small number of cues, especially when making split-second decisions (Blanchard, 1997). Animals typically filter sensory input for relevant cues (Bouskila & Blumstein, 1992; Ordiz *et al*., 2011), such as specific sounds (Bird & Emery, 2008), chemicals (Cooper, Jr., 1995; McCormick & Manassa, 2008), and visual cues such as movement (Ewert, 1974; Fleishman, 1986; Schulert & Dicke 2002), shadows (Cooper, Jr., 2009), proximity (Ydenberg & Dill, 1986) and looming (Schiff *et al*., 1962). By filtering this information, animals can reduce the amount of time they spend collecting information and increase time available for other behaviours, conferring direct fitness benefits (Dall *et al*., 2005; Lönnstedt & McCormick, 2011; Tvardìkova & Fuchs, 2011).

Due to the strong selection in favour of accurate information systems (Lima, 1998), especially when assessing predatory information (Abrams, 2000; Wisenden & Harter, 2001) risk-assessment is a particularly well-studied field of animal behaviour. As technology has improved, investigators have been able to manipulate cues more precisely to examine their role in mediating antipredatory behaviour (Carlile *et al*., 2006; Campbell *et al*., 2009), and this is particularly true of the use of video playback methods. The integration of computer

animation and video playback now means that it is possible to manipulate specific parameters such as morphology and movement (Rosenthal *et al*., 2004; Pruden & Uetz, 2004; Nelson *et al*., 2010). A great deal of work has been done examining visually-mediated antipredator behaviour in vertebrates (e.g. Carlile *et al*., 2006; King & Adamo, 2006; Ordiz *et al*. 2011; Smith & Evans, 2011), but information about anti-predatory behaviour in invertebrates is sparse by comparison.

Cephalopods are hailed as the "escape artists" of the invertebrate world (Anderson & Wood, 2001; Mather & Anderson, 2007). When initially threatened, cephalopods may manipulate their skin pigment cells to change colour, texture or pattern and blend in with their surroundings (Forsythe & Hanlon, 1997; King & Adamo, 2006; Hanlon *et al*., 2009; Mather *et al*., 2009). If a predator is undeterred or the predation event begins before the prey can camouflage itself, cephalopods can use their siphon to direct exhalant water in a jet-stream, propelling themselves away at high speed (Forsythe & Hanlon, 1997). This jetting is often combined with the expulsion of dark organic compounds which reduce visibility and mask chemical trails.

P. cordiformis shares its habitat with a number of fast-swimming pelagic predators such as the New Zealand fur seal (*Arctocephalus forsteri*), and must be ready to identify and evade these predators at only a moment's notice. However, the mechanisms underlying cephalopods' intricate antipredator responses (Forsythe & Hanlon, 1997; King & Adamo, 2006; Hanlon *et al*., 2009) are poorly understood. How do octopuses assess whether to flee from a predator, or whether to stay put and camouflage instead? What sensory systems interpret the environmental information and what cues are being discriminated in this risk assessment? Turbidity and topography make the use of visual cues in aquatic environments difficult at times (Lönnstedt & McCormick, 2011), and many aquatic social animals rely on chemical cues to mediate anti-predatory behaviour (Chivers & Smith, 1998; Alemadi & Wisenden, 2002; Brown, 2003). However, motion (Nakayama & Loomis, 1974; Regan & Beverley, 1984) and looming (Regan & Vincent, 1995; Carlile *et al*., 2006) are known to play important roles in risk-assessment within a wide variety of animals, including invertebrates (Schiff, 1965), amphibians (Schiff, 1965), reptiles (Carlile *et al*., 2006), birds (Wang & Frost, 1992) and primates (Schiff & Detwiler, 1979).

Here I examine the responses of *P. cordiformis* to digital animations of a sympatric predator, *Arctocephalus forsteri* (Anderson, 1999) in comparison to video clips of *A. forsteri*, and consider the role of size changes in risk assessment by *P. cordiformis*. In particular, I examine whether movement affected ventilation rate, appearance characteristics, state of alertness, and flinching.

Methods

Prior to testing, octopuses were captured and housed using the procedures discussed in Chapter 2. Following acclimation (Chapter 3), octopuses were presented with a series of video clips of a predator (the New Zealand fur seal, *Arctocephalus forsteri*). Videos were produced using Final Cut Express (Version No. 4.0.1) and were displayed to octopuses on a Samsung SyncMaster 2243 LCD computer screen. To reduce visual distraction, aquaria were covered on all sides by black polythene.

Three fur seals from the Kaikoura seal colony were photographed from the front, back, and side to be used in development of digitally animated clips. Due to photos from two of the seals being unsuitable, photos from only one of these individuals were used. Five types of clips were developed. The first three clips were digitally animated images of *A. forsteri*: 1. photographed from the front and manipulated to expand symmetrically over 15 seconds to give the appearance of looming; 2. photographed from the side and manipulated to move sideways across the frame over 15 seconds from right to left; 3. photographed from the rear and manipulated to contract symmetrically over 15 seconds to give the appearance of receding. Additionally, archival footage of a New Zealand fur seal was obtained from a BBC video archive (BBC motion clips) and used as a 25 second 'real seal' positive control, and a 30-second clip of a blue-coloured screen was used as a negative control. The colour of the negative control image was chosen by averaging 25 random samples of the ocean colour in the 'real seal' video, and was also used as the background for the three digitally animated clips. Relative fur seal size (Brunner, 1998) and movement speeds (Page *et al.*, 2006) in digitally animated clips were used to determine the appropriate visual angle of seal images on the display unit to simulate more realistic apparent motion.

After production, each clip was edited together in a randomised order (with replacement) for each individual octopus. Transitions between each clip were blended together using Final Cut Express to reduce sharp visual contrasts. To facilitate this, 1.5 seconds of the start/end frame was added to each video to allow for transition time without altering the length of each clip.

During a trial, the LCD screen was placed against one side of the tank and the negative control video was displayed. Octopuses were allowed to acclimate for 30 minutes during which time they were filmed to obtain baseline behaviour, before being shown a series of randomly assigned predator video clips.

Scoring of video playback data

Octopus behaviour was scored using JWatcher following procedures discussed in Chapter 3. The behaviours that were examined were ventilation, colouration, texture, body pattern, alertness, and flinching (see Chapter 2 for definitions). Flinching describes a behaviour where octopuses would rapidly compress their bodies downward, reducing their stature. After scoring, behavioural data was extracted from the raw JWatcher files into Excel and transferred to Prism (Version 5.0a) for graphing and analysis.

Analysis

Data consisted of count and proportion data, and were analysed and graphed in Prism 5.0a for Mac OS X. Being non-normally distributed, these data were analysed using Kruskal-Wallis tests and Mann-Whitney U tests. Where multiple comparisons were necessary, Bonferroni corrections were made to compensate for the inflation of Type I errors.

Results

Appearance

P. cordiformis did not adjust its colouration or body pattern in response to predator video playback (Fig. 6.1a-b), nor were there differences between sex, weight or damage levels (see tables 4.1-4.2). However, octopuses spent more time expressing smooth mantle texture in response to approaching ($U = 100.0$, $p = 0.003$; Fig. 6.1c) and laterally moving ($U = 108$, 0.006; Fig. 6.1c) seals than in response to the negative control video. These differences were

significant for males (approaching, U = 30.0, p = 0.017; laterally moving, U = 30.0, p = 0.017; Fig. 6.2), but not for females (see table 4.1).

Reactivity

All predator playbacks stimulated higher levels of alertness than observed in response to negative control playback (approaching, U = 84.0, p = 0.002; laterally moving, U = 95.5, p = 0.006; receding, U = 95.5, p = 0.006; real seal, U = 87.0, p = 0.003; Fig. 6.3a). However, there was no significant overall (treatment) difference in alertness levels between the different predator clips (Fig. 6.3a, see tables 4.3-4.4). Male *P. cordiformis* were more alert in response to approaching (U = 28.5, p = 0.019; Fig. 6.3b) and real seal (U = 28.5, p = 0.019; Fig. 6.3b) clips compared to negative control playback, while females were more alert in response to laterally moving (U = 9.50, p = 0.012; Fig. 6.3c) and receding (U = 9.50, p = 0.012; Fig. 6.3c) predators compared with negative control playback. Only one weight category (light-weight; W1) appeared to adjust their alertness in response to any video, being significantly more alert in response to approaching (U = 0.000, p = 0.010; Fig. 6.3d; table 4.4) and laterally moving (U = 0.000, p = 0.010; 6.3d; table 4.4) predators than in response to negative controls. Damage did not influence the level of alertness observed in response to any playback (see table 4.4).

Playback significantly influenced the number of times octopuses would flinch, with individuals flinching more in response to real seal playback than in response to approaching, laterally moving and receding seals (all: U = 110, p = 0.006; Fig. 6.4a) as well as in response to negative control playback (U = 118, p = 0.019; Fig. 6.4a). This effect was only significant in female *P. cordiformis* (see table 4.3), that flinched more in response to real seal playback than in response to approaching (U = 13.5, p = 0.030; Fig. 6.4b), laterally moving (U = 13.5, p = 0.030; Fig. 6.4b) and receding seals (U = 13.5, p = 0.030; Fig. 6.4b) as well as in response to negative control playback (U = 13.5, p = 0.030; Fig. 6.4b). Flinching expression did not differ with sex, weight or damage categories (see tables 4.3-4.4). Octopuses of medium mass (W2) octopuses flinched more in response to real seal playback than in response to approaching (U = 20.0, p = 0.035; Fig. 6.4c), laterally moving (U = 20.0, p = 0.035; 6.4c) and receding seals (U = 20.0, p = 0.035; 6.4c).

Ventilation was strongly modified by predator video playback, with significantly lower rates of ventilation in response to real seal playback than in response to approaching (U = 53.0, p = 0.0004; Fig. 6.5a), laterally moving (U = 13.0, p = <0.0001; 6.5a), and receding seal playbacks (U = 13.0, p = <0.0001; 6.5a). Ventilation rates were also significantly higher in response to approaching (U = 53.0, p = 0.003; Fig. 6.5a), laterally moving (U = 0.0000, p = <0.0001; 6.5a), and receding (U = 530 p = <0.0001; Fig. 6.5a) playbacks compared to the negative control. Male octopuses reduced their ventilation rates in response to real seal playback compared to approaching (U = 30.0, p = 0.041; Fig. 6.5b), laterally moving (U = 6.00, p = 0.002; Fig. 6.5b) and receding seals (U = 6.00, p = 0.002; Fig. 6.5b), and lowered their ventilation rates in response to negative control playback compared to laterally moving (U = 0.000, p = 0.001; Fig. 6.5b) and receding seals (U = 0.000, p = 0.001; Fig. 6.5b). Female *P. cordiformis* had lower ventilation rates in response to approaching seals compared to laterally moving (U = 3.00, p = 0.003; Fig. 6.5c) and receding (U = 5.00, p = 0.009; Fig. 6.5c) seals. Females had lower ventilation rates in response to real seal playback compared to approaching (U = 6.00, p = 0.007; Fig. 6.5c), laterally moving (U = 0.0000, p = 0.001; Fig. 6.5c) and receding seals (U = 1.00, p = 0.002; Fig. 6.5c), and lower ventilation rates in response to negative control playback compared to approaching (U = 0.000, p = 0.001; Fig. 6.5c), laterally moving (U = 0.000, p = 0.001; Fig. 6.5c), and receding (U = 0.000, p = 0.001; Fig. 6.5c) seal playback.

Octopuses from different weight categories adjusted their ventilation rates differently in response to video playback. Lightweight (W1) *P. cordiformis* lowered ventilation rates in response to real seal playback compared to approaching (U = 3.00, p = 0.042; Fig. 6.6a), laterally moving (U = 0.000, p = 0.020; Fig. 6.6a) and receding (U = 0.000, p = 0.012; Fig. 6.6a) seal playback, as well as in response to negative control playback, compared to laterally moving (U = 0.000, p = 0 0.030; Fig 6.6a) and receding (U = 0.000, p = 0 0.020; Fig 6.6a) seals. Medium-weight (W2) octopuses had lower ventilation rates in response to real seal playback compared to approaching (U = 17.0, p = 0.038; Fig. 6.6b), laterally moving (U = 2.00, p = 0.001; Fig. 6.6b), and receding (U = 3.00 p = 0.003; Fig. 6.6b) seal playbacks, and lowered ventilation rates in response to negative control playbacks compared to laterally moving (U = 0.000, p = 0.001; Fig. 6.6b) and receding (U = 0.000, p = 0.001; Fig. 6.6b) seal playbacks. W3 octopuses only lowered ventilation rates significantly in response to negative control playback compared to receding seal playbacks (U = 0.000, p = 0.020; Fig. 6.6c).

Damage category was also important in ventilatory responses in *P. cordiformis*. Undamaged and moderately damaged (D1 & D2) octopuses had significantly lower ventilation rates in response to real seal playbacks in comparison to approaching (D1, $U = 9.00$, $p = 0.040$; D2, $U = 5.500$, $p = 0.0500$; Fig. 6.7a-b), laterally moving (D1, $U = 0.000$, $p = 0.012$; D2, $U = 0.000$, $p = 0.008$; Fig. 6.7a-b) and receding (D1, $U = 0.000$, $p = 0.003$; D2, $U = 0.000$, $p = 0.012$; Fig. 6.7a-b) seals, and also had significantly lower ventilation rates in response to negative control playback compared to laterally moving (D1, $U = 0.000$, $p = 0.002$; D2, $U = 0.000$, $p = 0.002$; Fig. 6.7a-b) and receding (D1, $U = 0.000$, $p = 0.005$; D2, $U = 0.000$, $p = 0.012$; Fig. 6.7a-b) seals. Finally, heavily damaged (D3) octopuses significantly lowered ventilation rates in response to real seal playback compared to laterally moving ($U = 4.00$, $p = 0.054$; Fig. 6.7c) and receding ($U = 4.000$, $p = 0.054$; Fig. 6.7c) seals, as well as lowering ventilation rates in response to negative control playback compared to laterally moving and receding (both comparisons $U = 0.000$, $p = 0.008$; Fig. 6.7c).

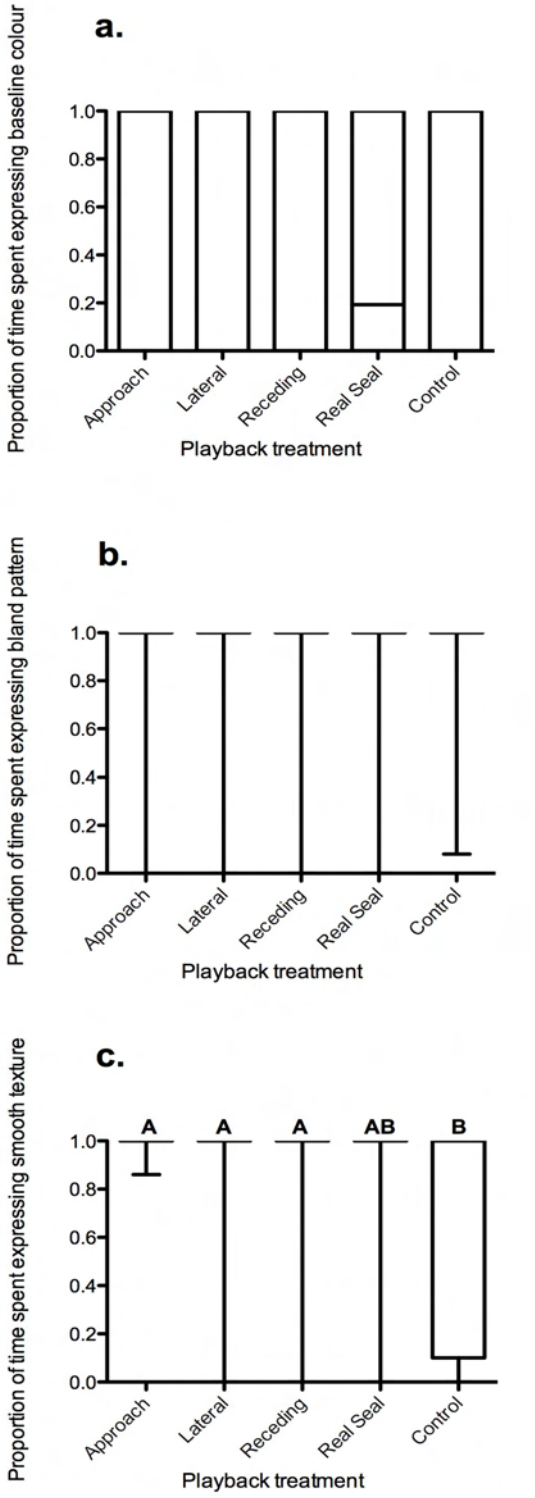

Figure 6.1: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent by *P. cordiformis* expressing appearance characteristics in response to video playback of digital seals approaching, moving laterally and receding, and to real seal footage and control playback (n = 19) (Kruskal-Wallis ANOVA). (a) Colouration (b) pattern (c) texture. Plotted points sharing letters are not significantly different.

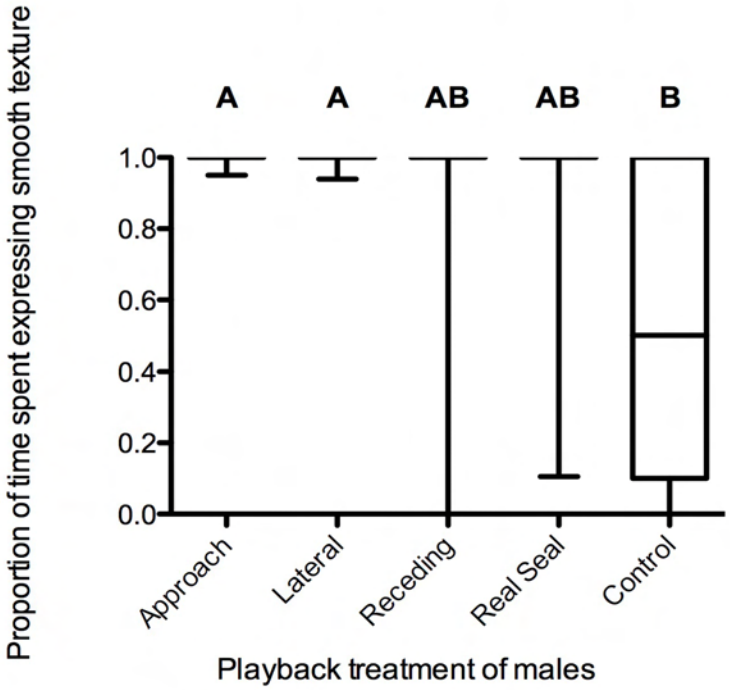

Figure 6.2: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent by male *P. cordiformis* expressing smooth texture in response to video playback of digital seals approaching, moving laterally and receding, and to real seal footage and control playback (n = 11) (Mann-Whitney U). Plotted points sharing letters are not significantly different.

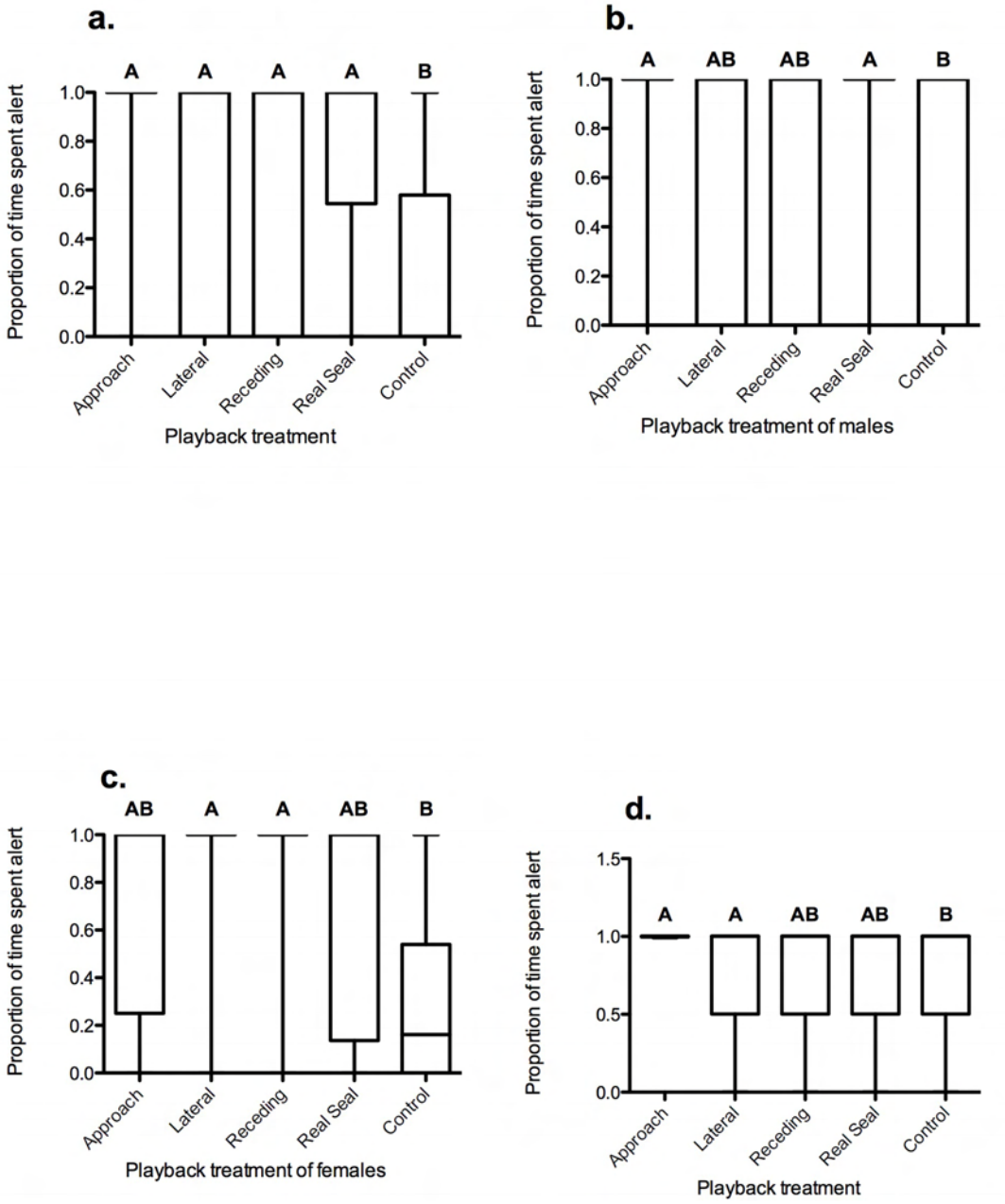

Figure 6.3: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the proportion of time spent by *P. cordiformis* being alert in response to video playback of digital seals approaching, moving laterally and receding, and to real seal footage and control playback (a) overall (n = 19), (b) males (n = 11), (c) females (n = 8), (d) Lightweight (W1; n = 5) (Kruskal-Wallis ANOVA). Plotted points sharing letters are not significantly different.

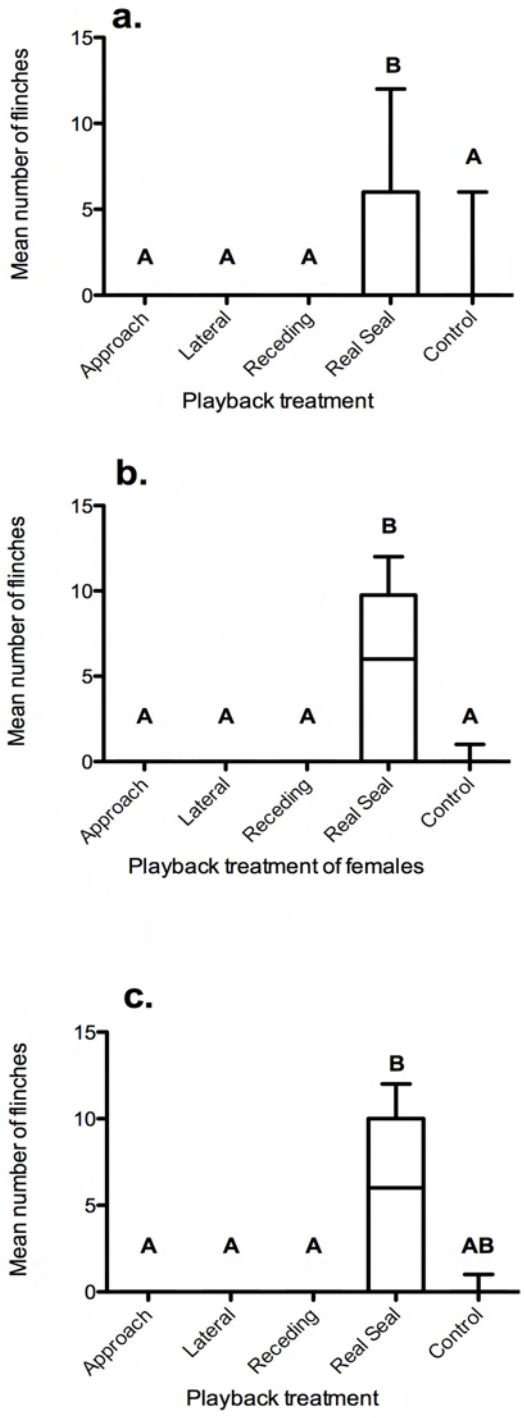

Figure 6.4: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the mean number of flinches expressed by *P. cordiformis* in response to video playback of digital seals approaching, moving laterally and receding, and to real seal footage and control playback (a) overall (n = 19), (b) females (n = 11), (c) medium weight (W2; n = 9), (Mann-Whitney). Plotted points sharing letters are not significantly different.

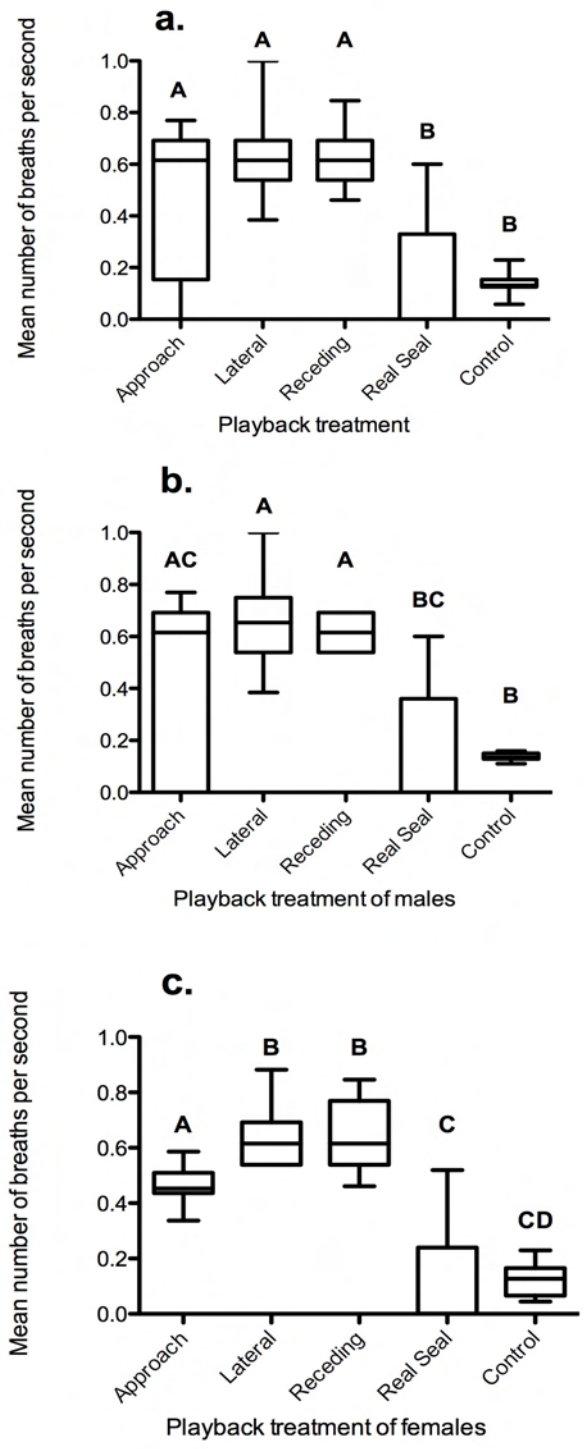

Figure 6.5: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the mean per-second ventilation rate of *P. cordiformis* in response to video playback of digital seals approaching, moving laterally and receding, and to real seal footage and control playback. (a) Overall comparison (n = 19), (b) males (n = 11) (c) females (n = 8) (Kruskal-Wallis ANOVA). Plotted points sharing letters are not significantly different.

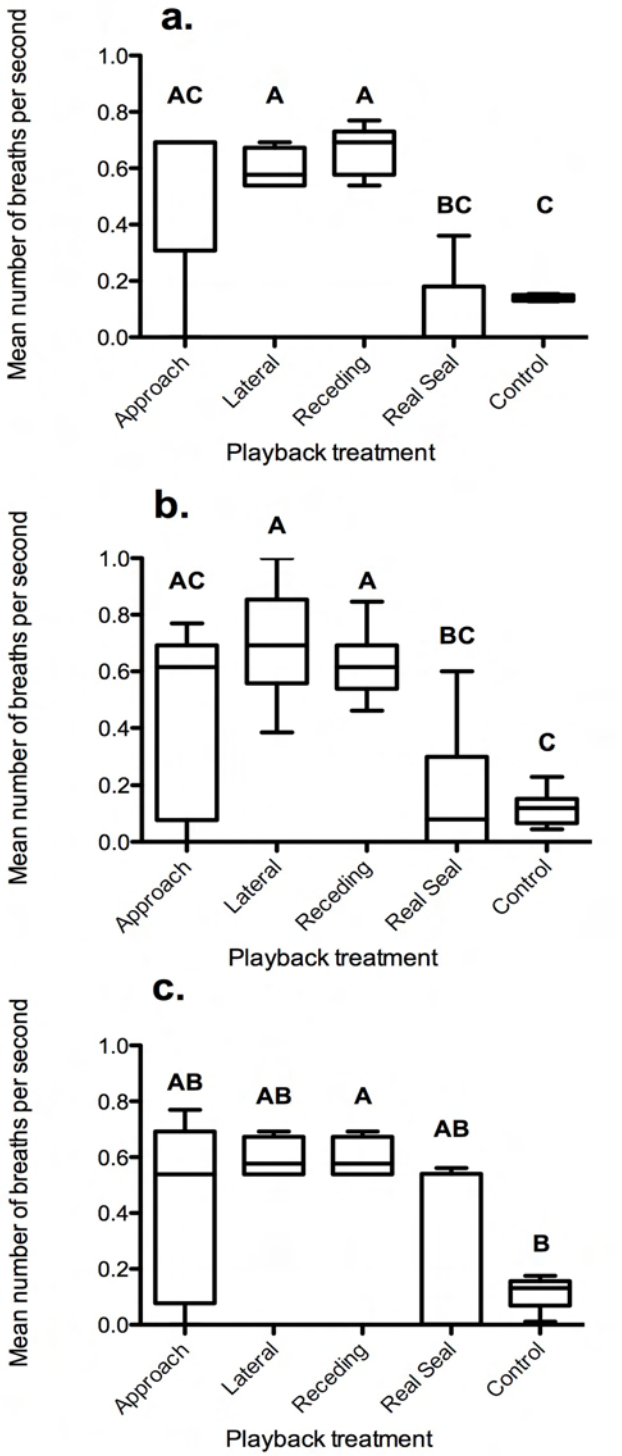

Figure 6.6: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the mean per-second ventilation rate of *P. cordiformis* in response to video playback of digital seals approaching, moving laterally and receding, and to real seal footage and control playback. (a) lightweight (W1; n = 5), (b) medium weight (W2; n = 9) (c) heavyweight (W3; n = 5) (Kruskal-Wallis ANOVA). Plotted points sharing letters are not significantly different.

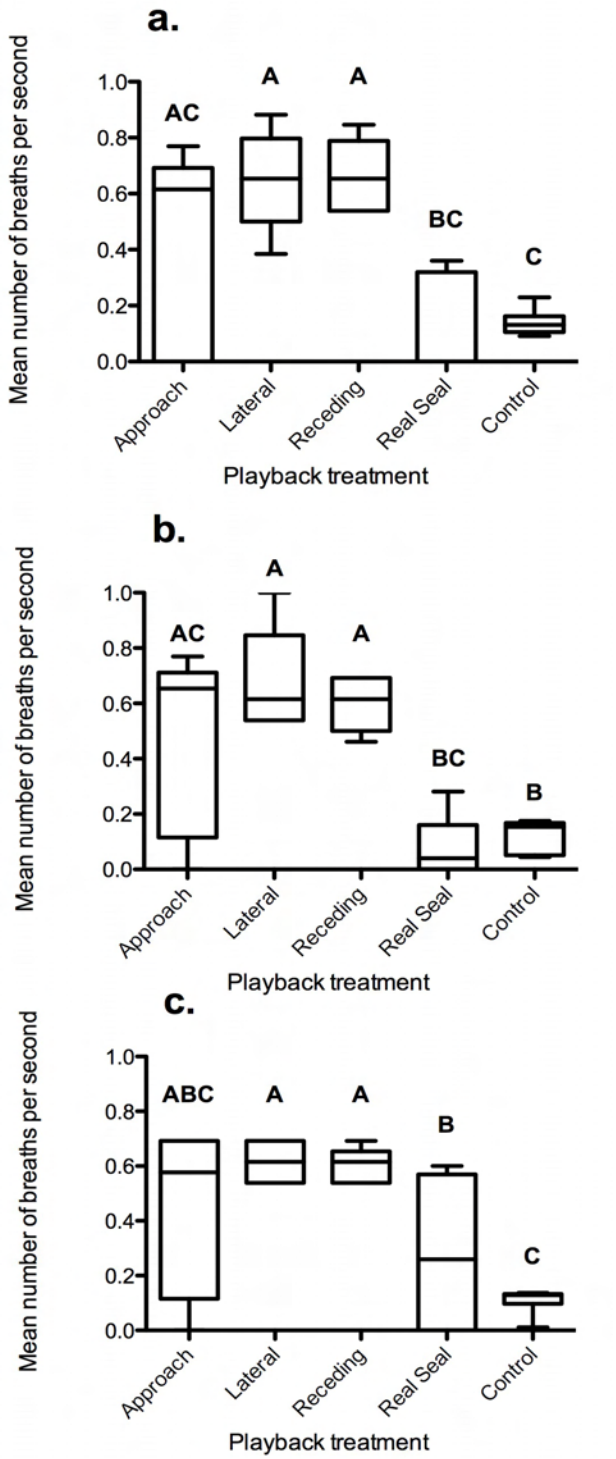

Figure 6.7: Boxplot (median and quartiles) with whiskers (minimum and maximum) of the mean per-second ventilation rate of *P. cordiformis* in response to video playback of digital seals approaching, moving laterally and receding, and to real seal footage and control playback. (a) undamaged (D1; n = 7), (b) moderately damaged (D2; n = 6) (c) heavily damage (D3; n = 6) (Kruskal-Wallis ANOVA). Plotted points sharing letters are not significantly different.

Discussion

Results from this study indicate that video playback of New Zealand fur seals can elicit antipredatory responses in *P. cordiformis*. While some behaviours were adjusted to different extents, others did not appear to be influenced by playback stimuli. Octopuses in this study were more alert in response to all video clips involving predators compared to the control clip. They lowered ventilation rates in response to real seal playback as well as the controls compared to all digitally animated clips, and flinched more in response to real seals compared to all digitally animated clips. Overall, these results suggest that *P. cordiformis* responds somewhat realistically to even rudimentary digital animations of predators, and may respond more realistically to unmanipulated footage in comparison to digitally manipulated static images.

Evidence from this study shows that octopuses were more alert in response to video footage featuring seals compared to control footage of a blank, blue screen. This attentiveness differed in males and females, and in light-weight octopuses. Male octopuses were more alert to approaching animated seals and to real seal footage compared to controls, while females were more attentive to laterally moving and receding animated seals. This suggests that male and female *P. cordiformis* have different sensitivities when it comes to responding to predators, supported by a heightened amount of flinching in response to real seal playback by females compared to all other video clips, whereas males did not flinch significantly more. Similarly, males adjusted their body texture in response to predator playback while no effect on appearance was observed in females. Differences in the way in which male and female octopuses adjusted their ventilation rates also suggests that each sex may be attending to subtly different cues. These differences may be explained by morphological sex differences in octopuses. The male reproductive organ is appendicised and sits at the end of one of the arms. Failure to evade a predator can potentially result in death for both sexes, and while octopuses may be able to sacrifice a limb via autotomy and escape alive (Maginnis 2006), if the male hectocotylus were removed during predation or via autotomy, this would entail either a total loss of fitness for males, or a significant loss in breeding ability while the limb is regenerated (if hectocotylus regeneration is even possible; Maginnis *et al*., 2006); a risk that is not shared with females.

Differences in response to predator playback were also observed in octopuses with different levels of weight and limb-damage. Lightweight (W1) subjects were more alert in response to approaching and laterally moving seals than to control clips, while no differences were observed in medium (W2) or heavyweight (W3) octopuses. Similarly, while all octopuses from all weight categories adjusted their ventilation rates in response to predator playback, the way in which each weight class responded differed to some extent. Most notably, heavy octopuses did not adjust their ventilation rates to real seal playback, suggesting that heavier (and potentially larger) octopuses may be a less viable target for foraging seals, perhaps due to better ability to defend themselves. Strong flinching in W2 octopuses in response to real seal playback where no such difference is observed in W3 octopuses supports this, although why W1 octopuses do not follow this trend is unclear.

Results also show that *P. cordiformis* adjusts ventilation rates differently depending on the amount of arm damage to which it has been subjected. Lightly (D1) and moderately (D2) damaged subjects responded similarly to all animated predator playbacks, reducing ventilation in comparison to both real seal playback and control footage. While ventilation rates of heavily damaged octopuses followed a similar pattern, they did not differ between approaching seals and the control playbacks. The reasons for this are unclear, and may be an artefact of analysis.

At first glance it may seem peculiar that octopuses respond with increased ventilation rates in response to digitally animated seals compared to control playbacks, but do not respond in the same way to real seal playback. Subjects would often freeze and hyperinflate the mantle in response to a threat (often seen when a person approached octopuses during early acclimation), and this behaviour was strongly associated with viewing real predator videos. Due to the potential costs associated with responding inappropriately to a given situation, escape responses in octopuses are probably context-specific, as seen in other animals (e.g. alligators, Smith *et al*., 1974; burrowing mammals, Smith *et al*., 1981). King and Adamo (2006) observed a similar response in threatened cuttlefish, suggesting that hyperinflation could assist in preparations for flight by jet propulsion. *Octopus vulgaris* (Wells, 1980; Wells *et al*., 1987) and the cuttlefish *Sepia officinalis* (Chichery, 1980) show similar responses to rapidly approaching objects, where subjects freeze and exhibit reductions in heart rate. Similar behavioural freezing and lowered ventilation has also been observed in vertebrates, so this behaviour is not phylogenetically distinct (Smith & Woodruff, 1980; Barham *et al*., 1985;

Cooke *et al*., 2003). However, some animals use different suites of behaviour in response to seemingly similar threat scenarios depending on the perceived threat, and these 'alternate responses' are observed in cephalopods (King & Adamo, 2006). In this study, approaching and laterally moving seals appear relatively distant compared to the real seal playback, which is immediately close to the camera. Thus octopuses may have raised their ventilation to prepare for the metabolic expenses of movement (Wells *et al*., 1987) and to prepare to escape relatively distant (animated) seals, but resorted to behavioural freezing in the face of the immediate threat of a near (real) seal.

The lack of difference between octopuses with different levels of limb damage is of special interest, due to strong evidence from other studies towards a taxonomically widespread tendency for antipredatory behaviour to be strongly mediated by learning (Griffin, 2004; Galef & Laland, 2005). Taken by itself, the lack of differentiation in response between undamaged and heavily damaged octopuses could suggest that *P. cordiformis* does not adjust its risk-assessment behaviour in response to predation. However, the context in which damage occurs may be important in mediating learned responses. Damage that has occurred as a result of intraspecific interactions might not strongly adjust behavioural responses to seals for instance. Antipredatory behavioural adjustments might also occur with respect to other cues. Aquatic environments such as the Kaikoura area, from which octopuses were collected can become highly turbid, with carbonate sediments from the algal community also lowering visibility (Reid *et al*., 2011). In such an environment, visual antipredatory cues would be essentially useless. In such conditions, and even in non-turbid conditions, it may be more effective for octopuses to respond strongly to pressure cues (Mooney *et al*., 2010). Similarly, André *et al*. (2011) have shown that cephalopods respond strongly to acoustic cues. Audio cues from nearby predators, such as cetaceans, in the proximity of an octopus could be used via 'eavesdropping' to reduce risk when visual cues are unavailable for use. Octopuses also utilise olfactory cues during foraging to overcome visual restrictions in such environments (Forsythe & Hanlon, 1997) and although chemical senses might not be helpful during attacks from predators once located, olfaction would allow octopuses to detect predators earlier and consider the threat of a potential predator in optimising their foraging decisions (Dall *et al*., 2005). The lack of the ability to induce realistic pressure, olfactory and acoustic cues is a clear drawback of the current study, and a consideration for future efforts.

Low levels of change to visual characteristics observed in this study support the hypothesis that visual cues do not induce the full suite of antipredatory behaviour in *P. cordiformis*. Subjects adjusted texture to a small extent, but no significant differences in pattern or colouration were observed during any trial. Cephalopods are famous for their capacity to manipulate the appearance of their bodies while hunting (Forsythe & Hanlon, 1997) and avoiding prey (Hanlon *et al*., 1999; Messenger, 2001) to break a viewer's search image (Curio, 1976), and it seems improbable that *P. cordiformis* would not make use of this capacity to respond to impending predation. However, if the visual acuity of seals may be low while immersed in water that is turbulent or high in particulate matter (Reid *et al*., 2011), they may utilize other senses to hunt in place of vision, in which case crypsis would not be an effective mechanism of predator avoidance. Certainly one would not expect significant selective pressure for camouflage as a defence against echolocating cetaceans, and octopuses are indeed sensitive to such sounds (Mooney *et al*., 2010; André *et al*., 2011). This lack of appearance response may indicate that deimatic displays in *P. cordiformis* are mediated not by visual information, but by other cues such as pressure and sound.

To realistically reproduce motion it is necessary to capture realistic and biologically relevant movement patterns (Woo & Riecau, 2008). In a study of *Anolis* lizards, Fleishman (1988) showed that "movement characterised by abrupt changes in velocity is more effective in eliciting an orienting response…than smooth sinusoidal motion". In this study, the movement of digitally animated seals was explicitly linear, while the motion of the predator in the 'real seal' video clip showed abrupt changes both in velocity and in direction. Responses to the 'real seal' video thereby support results from Fleishman (1988). In conjunction with automated computer software, Watanabe and Troje (2006) recreated biologically realistic motion in an animated pigeon using high-speed cameras, which tracked reflective markers on real pigeons. However, without the use of such technology, creating realistic motion can be extremely difficult. This may explain some of the differences observed between the animated playback clips and the real clip used in this study (for example, reduced ventilation and increased flinching in response to real seals compared to animated clips). By using more complex animations, realistic motion patterns that might stimulate more realistic responses among *P. cordiformis* may be achieved.

Escape mechanisms such as jetting were not observed in this trial, although it is not certain whether this was due insufficient stimulation from the predator playback. The context under

which octopuses are responding to potential threats from predators in these trials is also important to consider. Octopuses are known to have excellent spatial memory (Mather, 1991; Boal *et al*., 2000), and these trials took place in a space-limited environment in which refugia were removed prior to playback. Prey often respond to risk by increasing their use of refugia (Sih, 1986; Cooper Jr, 1998; Lòpez, 2005). It is possible that *P. cordiformis* did not resort to escape mechanisms because they were aware that there was no place to escape to, due to the limited space and removal of dens from the experimental arena.

Conclusions & Future Directions

The variable changes in the different behaviours of *P. cordiformis* indicate that visual cues are not sufficient to stimulate a full suite of antipredatory responses. As suggested by Lönnstedt and McCormick (2011), prey that respond to inappropriate cues when assessing risk spend more time performing defensive behaviours at the expense of other behaviours that can benefit fitness. Prey should therefore respond to the cues that best predict predation risk, which may not necessarily be visual cues, in the case of *P. cordiformis*. In aquatic environments subject to intermittent high turbidity it may be more beneficial to respond to reliable pressure (Komak *et al*., 2005; Mooney *et al*., 2010), auditory (Womble *et al*., 2007) or olfactory cues (McCormick & Manassa, 2008) than to assess risk using visual cues in a visually obscured environment.

Reductions in ventilation indicate that video playback was sufficient to elicit preparatory antipredator behaviour, but not to elicit full fight-or-flight responses from *P. cordiformis*. This may be due to methodological shortcomings, such as sub-optimal illumination cues or computer animation design, or it may reflect that *P. cordiformis* was not sufficiently threatened by the appearance of a fur seal. Tvardíkova & Fuchs (2011) noted that the proximity of a predator does not necessarily determine the risk of a predation event. Predation risk can differ depending on the behaviour of individual species of predator (Curio *et al*., 1983). Testing *P. cordiformis* and other octopuses for their responses to a more direct threat in the field to obtain realistic predator-prey interactions against which the results of this study seem like an important consideration.

Chapter 7: General Discussion

Synopsis

This study has investigated some of the behavioural aspects of *Pinnoctopus cordiformis* in the contexts of adaptation to captive environments, behavioural sleep patterns, and visual responses to digital video playback. Little is known about the behaviour of *P. cordiformis*, and although parallels can be drawn from well known octopuses such as *Octopus vulgaris* (e.g. Fiorito *et al*., 1990; Mather & O'Dor, 1991; Fiorito & Scotto, 1992; Hochner *et al*., 2003; Brown *et al*., 2006; Anderson *et al*., 2008; Gutnick *et al*., 2011), *O. cyanea* (e.g. Yarnall, 1969; Forsythe & Hanlon, 1997; Mather & Mather, 2004) and *O. bimaculoides* (e.g. Boal *et al*., 2000; Sinn *et al*., 2001; Hvorecny *et al*., 2007), this study bolsters knowledge about a native New Zealand species, and raises interesting considerations regarding cephalopod behaviour in a broader evolutionary context. These results indicate that *P. cordiformis* is robust in its response to the stresses associated with capture and captivity, is responsive to video playback trials using both digitally constructed and filmed animals presented on an LCD screen, and is able to tolerate sleep deprivation for at least a 12 hour duration. The latter of these observations does not align with similar studies by Brown *et al*. (2006), which found homeostatic regulation in quiescent behaviour of *O. vulgaris* following 12-hour sleep deprivation. This highlights the interspecific diversity of octopus behaviour and the need for caution when making assumptions about individual species based on previously established material from studies of related species.

By the same token, the attendance of *P. cordiformis* to video playback of visual stimuli differing in motion and shape corresponds to previous observations of *O. tetricus* responses to video playback (Pronk *et al*., 2010). This is the first demonstration that *P. cordiformis* attend to both motion and shape cues during predator-prey interactions. Both of these visual cues elicited context-relevant behavioural responses, indicating that the speculative chemotactic foraging believed to be typical of octopuses (Chase & Wells, 1986; Hanlon & Messenger, 1996; Forsythe & Hanlon, 1997) is either facilitated by, or occurs in addition to, attendance to visual cues.

Sex, weight and the limb condition of octopuses all appeared to influence certain aspects of *P. cordiformis'* behaviour (for example: levels of attention and rates of ventilation in response to video playback, and variable grooming responses during acclimation). However, the overall influence of each of these traits was relatively low across all four experiments, and it appears that there are not strong differences in behaviour between male and female octopuses, or between octopuses of the weight categories and limb conditions examined in this study. This does not preclude behavioural differences in senescent and/or young and larval octopuses (Messenger, 2001; Anderson *et al*., 2002; Mather, 2008). These factors were not studied here, in part because one of the disadvantages of working with live-caught octopuses is the difficulty associated with determining the age of each individual without post-mortem measurement of beak size (Perales-Raya *et al*., 2010). However, this can be overcome by using lab-reared octopuses, and the robustness of *P. cordiformis* observed during this acclimation and captivity suggests that this species may be a viable subject for lab-rearing experiments.

The function of sleep in regulating cognitive function in other animals has been demonstrated by numerous researchers (Hobson, 2005; Siegel, 2005; Capellini *et al*., 2008), although this is still contested by others, who suggest that sleep functions primarily for metabolic and maintenance purposes (see McNamara *et al*., 2009). The findings of the current study add some support to observations made by Brown *et al*. (2006). While here homeostatic regulation in quiescent behaviour was not found, these results may suggest that octopus rest states are analogous to mammalian NREM cycles, where subjects have heightened arousal thresholds and stereotyped behavioural quiescence (McNamara *et al*., 2009). Part of the criteria for true "sleep" in animals is homeostatically regulated rest behaviour. While this was observed during sleep deprivation trials, the behaviours affected (grooming and luring) do not clearly relate to sleep. As such rather than being a true effect of sleep deprivation it is possible that these results may be confounded by stress. Controlling for stress and treatment effects in trials where subjects must be kept awake via mechanical stimulation is likely to be extremely difficult, if not impossible, and it is likely that pharmacological knockout studies, coupled with neurological measurement may be a more viable avenue to continue investigation.

Aside from difficulties controlling for stress and distinguishing sleep responses from behavioural quiescence in the sleep deprivation experiment, methodological constraints are unlikely to have heavily impacted the results in this thesis. This is especially true for the

acclimation study, although it is important to note that a lack of enrichment in the aquaria during all studies (including acclimation) has the potential to generate behaviour that does not align with what might be seen under more natural environments (Ődberg, 1987; Rehling, 2000; Anderson & Wood, 2001; Wickens & Heleski, 2010). Furthermore, a number of factors relating specifically to video playback presentation are worth consideration and may strengthen conclusions drawn in subsequent playback studies.

Methodological considerations

The lack of changes to appearance characteristics and behaviours such as locomotion in response to predator playback may indicate that behaviour in *P. cordiformis* is mediated not only by visual information, but also by other cues such as pressure and sound. Video images can generate realistic responses in a wide range of taxa (Oliveira *et al*., 2000), and although video playback is no longer a new technique in behavioural science (dating back to the 1960s; Bird & Emery, 2008), disparities between the human display devices and non-human vision can confound what appear to be straightforward image or video presentations (Fleishmann *et al*., 1998; D'Eath, 1998). Shadows, specular reflections, brightness, luminance and contrast can all factor in how cues are viewed (Zeil, 2000; Rosenthal, 2000; Oliveira *et al*., 2000) and such conditions should ideally match natural conditions (Endler, 1993; Oliveira *et al*., 2000). Furthermore, flicker fusion rate (D'Eath, 1998), depth perception, motion parallax, spatial resolution, and colour perception (Cutthill *et al*., 2000; Fleishman & Endler, 2000; Oliveira *et al*., 2000; Zeil, 2000; Riecau & Giraldeau, 2009), as well as non-visual cues such as vibration and sound (Mooney *et al*., 2010; André *et al*., 2011) should ideally be tightly controlled to maximise realism and minimise behavioural artefacts arising from physiological disparities between human and non-human information systems. Given appropriate consideration of these potentially confounding cues, video playback and computer animation have a tremendous capacity for controlled manipulation of specific parameters of a desired stimulus (Oliveira *et al*., 2010).

The use of polarised light in cephalopod communication with predators, prey and conspecifics (Shashar & Cronin, 1996; Shashar *et al*., 2000) is particularly worth noting in the context of video playback, as Zeil (2000) suggested that contemporary display units cannot reproduce natural polarisation reflections. Furthermore, Pronk *et al*. (2010) noted that LCD monitors use

polarising filters that polarise pixels in different ways. This may detract from stimulus realism, although the use of LCD screens did not appear to prevent *O. tetricus* from responding realistically in the Pronk *et al*. (2010) study. Whether polarisation artefacts have influenced *P. cordiformis* behaviour in this study is unclear, and this is certainly an avenue of inspection for future video playback studies testing cephalopod behaviour. Here, the flicker fusion problems highlighted by D'Eath *et al*. (1998) were mitigated by the use of LCD's, which updated each pixel instead of refreshing the entire screen (Bird & Emery, 2008). Nevertheless, it is not possible to rule out that octopuses may instead have perceived some degree of image blurring as stimuli changed position on the screen, due to the rates of pixel updating. However, this is not likely, as pixels were updated every four milliseconds and octopuses have eyes adapted for relatively low-light environments and are consequently unlikely to have very fast photoreceptors.

Although octopuses are thought to be colour blind (Messenger, 1977), inappropriate rendering of specific light frequencies may still create false cues about the frequency and amount of light reflecting off the individual (Zeil, 2000; Oliveira *et al*., 2000; Pronk *et al*., 2010). A study by Kawamura *et al*., (2001) confirmed results from Messenger's (1977) work on *O. vulgaris* but also found that another species, *Octopus aegina* was capable of some degree of colour perception. It is not certain at this point whether *P. cordiformis* can see in colour, and whether colour artefacts produced by a human-attuned visual display system may have degraded the quality of the visual image being presented to subjects in these trials. Future studies may need to resolve this question by utilising well-controlled playback experiments where specific wavelengths are manipulated concurrently with behavioural and EEG investigation.

Conclusion and future directions

Acclimation and captivity can be critical in obtaining scientifically valid results, yet preventing and mitigating the effects of stress in an experimental scenario can be costly, and difficult to prevent. This may contribute to the tendency for researchers to focus on a handful of studied species where baseline behaviour is already established. Such a focus on a limited range of representatives from any taxon can, however lead to misinformation about that taxon when focal species possess traits that are species-specific and not shared among the wider

group. This study begins to establish baseline observations about the behavioural patterns of a native New Zealand octopus, *P. cordiformis*, in efforts to expand current knowledge on cephalopod behaviour, which is classically dominated by only a few well-studied species (e.g. *Octopus vulgaris*, *O. cyanea* and *O. bimaculoides;* Yarnall, 1969; Fiorito, *et al.*, 1990; Mather & O'Dor, 1991; Fiorito & Scotto, 1992; Forsythe & Hanlon, 1997; Boal *et al*., 2000; Sinn, *et al.* 2001; Hochner *et al*., 2003; Mather & Mather, 2004; Brown *et al*., 2006; Hvorecny *et al*., 2007; Anderson, *et al.*, 2008; Gutnick *et al*., 2011). The robust responses of *P. cordiformis* to acclimation and captivity in this study highlight the species' viability for laboratory-based investigation, and offer opportunities for researchers to examine octopus behaviour outside the realm of better-studied species. Results from these experiments highlight several departures from the conclusions of previous work on other octopus species (e.g. Brown *et al*., 2006), which reinforces the need for taxonomical diversity in behavioural studies.

The results from sleep deprivation trials conducted on *P. cordiformis* in this study support the presence of NREM-like rest states in octopuses, as shown by Brown *et al*. (2006). However, these results also suggest that *P. cordiformis* does not fulfil all of the established criteria for "true sleep" (McNamara *et al*., 2009). This is unsurprising, and echoes the tremendous diversity of sleep patterns observed in vertebrates, which vary according to ecotype (Capellini *et al*., 2008; 2009) and life stage (McNamara *et al*., 2009). The literature examining sleep in invertebrate species is sparse compared with that on vertebrates (McNamara *et al*., 2009). Yet the high level of octopus intelligence (Mather, 2008), and neural parallels to vertebrates that are being discovered in cephalopods (Bullock & Başar, 1984; Hochner *et al*., 2003; Brown *et al*., 2006), suggest that cephalopod brains are subject to evolutionarily parallel differentiation of neural substrates to facilitate function. Rattenborg *et al*. (2009) suggested that "the convergent evolution of homeostatically regulated SWS (slow wave sleep) in mammals and birds was directly linked to the convergent evolution of large, heavily interconnected brains capable of performing complex cognitive processes in each group". REM sleep in many young and developing mammals is expressed at much higher levels than in adults, supporting the suggestions by some researchers that sleep state is important in brain development (Siegel, 2005). If there are strong parallels in the evolution of the brains of cephalopods and higher vertebrates, examining the expression of low-voltage REM vs. high-amplitude slow wave NREM activity in octopuses across age gradients would be a viable method of testing the convergence of octopus brain and sleep structure on those of higher vertebrates. Forging together current video playback and animation technology with recent neurological advances

will enable researchers to examine the brains of the most intelligent invertebrates in a comparative context, in turn helping to elucidate the process by which intelligence evolves in all animals.

Although behavioural studies are very valuable, the examination of *P. cordiformis* acclimation, sleep deprivation and video playback responses would all benefit from a focused examination of physiological aspects, using more robust indicators of stress and neural activity. This, however, would require a much more extensive and potentially invasive sampling effort, which is not currently achievable. Instantaneous blood sampling and cannulation are unsuitable due to restrictions on mobility imposed by cannulae and the tendency for octopus stress hormone levels to spike during handling (Malham *et al.*, 2002). In the event that acclimation, for instance, has not occurred within the three-day timeframe that was set in this study, future studies should use a longer acclimation period to examine long-term effects, integrating blood-chemistry assessment as technology permits. The need for further metrics by which sleep deprivation effects are tested could also be addressed by examining the influence of sleep deprivation on parameters such as heart-rate, ventilation, blood-hormone levels (Siegel, 2008), as well as the use of EEG (Brown *et al.*, 2006) to provide a clearer indication of the potential influence (or lack) of sleep deprivation in *P. cordiformis*.

Finally, the lack of realistic motion, pressure, acoustic and olfactory cues in the current studies is a clear drawback, and a consideration for future efforts. In this study, the movement of digital seals was linear, while the motion of the predator in the archival 'real seal' clip changed velocity and direction abruptly. Realistic motion requires the capture of biologically relevant movement patterns (Woo & Riecau, 2008). Furthermore, an examination of additional sensory modalities would allow researchers to establish a hierarchy of sensory inputs, and also to test the ways in which these sensory modalities interact. It is apparent that video playback can elicit responses from octopuses, and consequently refining these techniques will provide researchers with a means to test octopuses in a broad range of hypotheses in different areas, including mate selection (e.g. Clark & Uetz, 1993; Rosenthal *et al.*, 1996; Witte & Klink, 2005), intraspecific aggression (Rowland *et al.*, 1995; McKinnon & McPhail, 1996), communication (Hanlon & Messenger, 1996; Messenger, 2001) and learning (Boal *et al.*, 2000; Mather, 2008b). Marrying video playback to neural imaging techniques (e.g. Brown *et al.*, 2006) enables experimenters to manipulate stimulus parameters with high

specificity and simultaneously to effectively map response activity to specific regions of the brain, revealing the differences and parallels in the structure and function of cephalopod brains with those of vertebrates.

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Appendix 1: Acclimation

Table. 1.1 P values and test statistics for the effect of (1) day (n= 16), (2) day delineated by sex (n_{males} = 9; $n_{females}$ = 7), and (3) sex (Dpoled; pooled values across all days; n_{males} = 9; $n_{females}$ = 7) on the (a) alertness levels, (b) handling activity and (c) luring behaviour of *P. cordiformis*. Bold and italicised test statistics indicate the use of Kruskal-Wallis ANOVA and Mann-Whitney U test respectively.

		Day (Overall)	Sex (Day)		Sex (DPooled)
			Male	Female	
Alert	P	0.670	0.900	0.740	0.84
	H/*U*	**0.800**	**0.220**	**0.600**	*29.0*
Handling	P	0.770	0.560	0.320	0.35
	H/*U*	**0.540**	**1.15**	**2.26**	*22.0*
Luring	P	0.740	0.910	0.670	0.61
	H/*U*	**0.590**	**0.170**	**0.800**	*26.0*

Table. 1.2 P values and test statistics (Kruskal-Wallis ANOVA) for the effect of (1) day delineated by weight ($W1_n = 4$; $W2_n = 7$; $W3_n = 5$), (2) weight (Dpooled; pooled values across all days; $W1_n = 4$; $W2_n = 7$; $W3_n = 5$), (3) day delineated by damage ($D1_n = 6$; $D2_n = 6$; $D3_n = 4$) and (4) damage (Dpooled; pooled values across all days; $D1_n = 6$; $D2_n = 6$; $D3_n = 4$) on the (a) alertness levels, (b) handling activity and (c) luring behaviour of *P. cordiformis*

		Weight (Day)			Weight (DPooled)	Damage (Day)			Damage (DPooled)
		W1	W2	W3		D1	D2	D3	
Alert	P	0.595	0.943	0.629	0.136	0.339	0.402	0.500	0.390
	H	1.08	0.117	0.929	3.99	2.16	1.82	1.39	1.88
Handling	P	0.668	0.517	0.991	0.51	0.728	0.331	0.839	0.151
	H	0.808	1.28	0.183	1.345	0.635	2.21	0.351	3.78
Luring	P	0.841	0.994	0.683	0.635	0.297	0.911	0.874	0.943
	H	0.346	0.011	0.762	0.908	2.43	0.187	0.269	0.118

Table. 1.3 P values and test statistics for the effect of (1) day (n= 16), (2) day delineated by sex (n_{males} = 9; $n_{females}$ = 7), and (3) sex (Dpoled; pooled values across all days; n_{males} = 9; $n_{females}$ = 7) on the (a) hiding and (b) grooming behaviour of *P. cordiformis*. Bold and italicised test statistics indicate the use of Kruskal-Wallis ANOVA and Mann-Whitney U test respectively.

		Day (Overall)	Sex (Day)		Sex (DPooled)
			Male	Female	
Hiding	P	0.929	0.540	0.927	0.397
	H/*U*	**0.148**	**1.23**	**0.151**	*23.0*
Grooming	P	0.450	0.251	0.971	0.837
	H/*U*	**1.60**	**2.76**	**0.059**	*29.0*

Table. 1.4 P values and test statistics (Kruskal-Wallis ANOVA) for the effect of (1) day delineated by weight ($W1_n = 4$; $W2_n = 7$; $W3_n = 5$), (2) weight (Dpooled; pooled values across all days; $W1_n = 4$; $W2_n = 7$; $W3_n = 5$), (3) day delineated by damage ($D1_n = 6$; $D2_n = 6$; $D3_n = 4$) and (4) damage (Dpooled; pooled values across all days; $D1_n = 6$; $D2_n = 6$; $D3_n = 4$) on the (a) alertness levels, (b) handling activity and (c) luring behaviour of *P. cordiformis*.

		Weight (Day)			Weight (DPooled)	Damage (Day)			Damage (DPooled)
		W1	W2	W3		D1	D2	D3	
Hiding	P	0.830	0.752	0.226	0.635	0.970	0.505	0.549	0.593
	H	0.372	0.571	2.97	0.908	0.062	1.37	1.20	1.05
Grooming	P	0.783	0.407	0.795	0.357	0.639	0.964	0.234	0.745
	H	0.488	1.80	0.459	2.06	0.896	0.074	2.904	0.588

Table. 1.5 P values and test statistics for the effect of (1) day (n= 16), (2) day delineated by sex (n_{males} = 9; $n_{females}$ = 7), and (3) sex (Dpoled; pooled values across all days; n_{males} = 9; $n_{females}$ = 7) on the (a) walking, (b) climbing and (c) swimming behaviour of *P. cordiformis*. Bold and italicised test statistics indicate the use of Kruskal-Wallis ANOVA and Mann-Whitney U test respectively.

		Day (Overall)	Sex (Day)		Sex (DPooled)
			Male	Female	
Walking	P	0.896	0.791	0.491	0.299
	H/*U*	**0.219**	**0.469**	**1.43**	*21.0*
Climbing	P	0.594	0.504	0.287	0.837
	H/*U*	**1.04**	**1.37**	**2.50**	*29.0*
Swimming	P	0.268	0.479	0.453	0.426
	H/*U*	**2.63**	**1.47**	**1.58**	*23.5*

Table. 1.6 P values and test statistics (Kruskal-Wallis ANOVA) for the effect of (1) day delineated by weight ($W1_n = 4$; $W2_n = 7$; $W3_n = 5$), (2) weight (Dpooled; pooled values across all days; $W1_n = 4$; $W2_n = 7$; $W3_n = 5$), (3) day delineated by damage ($D1_n = 6$; $D2_n = 6$; $D3_n = 4$) and (4) damage (Dpooled; pooled values across all days; $D1_n = 6$; $D2_n = 6$; $D3_n = 4$) on the (a) walking, (b) climbing and (c) swimming behaviour of *P. cordiformis*.

		Weight (Day)			Weight (DPooled)	Damage (Day)			Damage (DPooled)
		W1	W2	W3		D1	D2	D3	
Walking	P	0.841	0.927	0.894	0.618	0.853	0.805	0.986	0.344
	H	0.346	0.151	0.225	0.962	0.319	0.433	0.029	2.13
Climbing	P	0.491	0.316	0.752	0.057	0.541	0.312	0.460	0.909
	H	1.42	2.30	0.570	1.00	1.23	2.33	1.55	3.31
Swimming	P	0.449	0.239	0.971	0.562	0.719	0.232	0.445	0.235
	H	1.60	2.86	0.059	3.00	0.660	2.92	1.62	2.90

Table. 1.7 P values and test statistics for the effect of (1) day (n= 16), (2) day delineated by sex (n_{males} = 9; $n_{females}$ = 7), and (3) sex (Dpooled; pooled values across all days; n_{males} = 9; $n_{females}$ = 7) on the (a) colour, (b) pattern and (c) texture expression in *P. cordiformis*. Bold and italicised test statistics indicate the use of Kruskal-Wallis ANOVA and Mann-Whitney U test respectively.

		Day (Overall)	Sex (Day)		Sex (DPooled)
			Male	Female	
Colour	P	3.61	0.304	0.905	0.229
	H/*U*	**2.04**	**2.38**	**0.200**	*21.0*
Pattern	P	1.96	0.312	0.297	0.299
	H/*U*	**3.26**	**2.33**	**2.43**	*21.0*
Texture	P	0.156	0.495	0.274	0.174
	H/*U*	**3.72**	**1.41**	**2.59**	*18.0*

Table. 1.8 P values and test statistics (Kruskal-Wallis ANOVA) for the effect of (1) day delineated by weight ($W1_n = 4$; $W2_n = 7$; $W3_n = 5$), (2) weight (Dpooled; pooled values across all days; $W1_n = 4$; $W2_n = 7$; $W3_n = 5$), (3) day delineated by damage ($D1_n = 6$; $D2_n = 6$; $D3_n = 4$) and (4) damage (Dpooled; pooled values across all days; $D1_n = 6$; $D2_n = 6$; $D3_n = 4$) on the (a) colour, (b) pattern and (c) texture expression in *P. cordiformis*.

		Weight (Day)			Weight (DPooled)	Damage (Day)			Damage (DPooled)
		W1	W2	W3		D1	D2	D3	
Alert	P	0.595	0.543	0.850	0.817	0.641	0.366	0.735	0.607
	H	1.04	1.22	0.326	0.407	0.889	2.01	0.615	1.00
Handling	P	0.551	0.386	0.089	0.222	0.960	0.108	0.443	0.119
	H	1.192	1.90	4.84	3.02	0.082	4.46	1.63	4.25
Luring	P	0.668	0.427	0.51	0.169	0.529	0.692	0.50	0.142
	H	0.808	1.70	1.35	3.56	1.28	0.737	1.39	3.90

Appendix 2: Sleep Deprivation

Table. 2.1 P values and test statistics (Mann-Whitney U test) for the effect of (1) sleep deprivation (n= 16) and (2) sleep deprivation delineated by sex (n_{males} = 4; $n_{females}$ = 4-6 on the (a) sleeping, (b) luring and (c) grooming behaviour of *P. cordiformis*. * indicates a significant effect.

		Sleep Deprivation	Sex	
			Male	Female
Sleeping	P	0.829	0.610	0.886
	U	37.0	9.00	7.00
Luring	P	0.050*	0.762	0.057
	U	18.0	18.0	1.00
Grooming	P	0.050*	0.915	0.029*
	U	18.0	12.0	0.000

Table. 2.2 P values and test statistics (Mann-Whitney U test) for the effect of (1) sleep deprivation delineated by weight ($W1_n = 4$; $W2_n = 7$; $W3_n = 5$), (2) Sleep deprivation delineated by damage ($D1_n = 3$; $D2_n = 3\text{-}5$; $D3_n = 3$) on the (a) sleeping, (b) luring and (c) grooming behaviour of *P. cordiformis*.

		Weight			Damage		
		W1	W2	W3	D1	D2	D3
Sleep	P	0.700	0.786	0.700	0.250	0.700	1.00
	U	3.000	6.00	3.00	3.00	3.00	4.00
Luring	P	0.100	0.393	0.700	0.7000	0.786	1.000
	U	0.000	4.000	3.000	3.000	6.000	4.000
Grooming	P	0.100	1.00	0.40	0.127	0.700	0.700
	U	0.000	7.00	2.00	2.00	3.00	3.00

Table. 2.3 P values and test statistics (Mann-Whitney U test) for the effect of (1) sleep deprivation (n= 16) and (2) sleep deprivation delineated by sex (n_{males} = 4-6; $n_{females}$ = 4 on the (a) walking, (b) climbing and (c) swimming behaviour of *P. cordiformis*.

		Sleep Deprivation	Sex	
			Male	Female
Walking	P	0.422	1.00	0.460
	U	30.5	12.00	5.00
Climbing	P	0.424	0.610	0.245
	U	30.5	9.00	3.50
Swimming	P	0.246	0.610	0.460
	U	26.5	9.00	5.00

Table. 2.4 P values and test statistics (Mann-Whitney U test) for the effect of (1) sleep deprivation delineated by weight ($W1_n = 4$; $W2_n = 7$; $W3_n = 5$), (2) Sleep deprivation delineated by damage ($D1_n = 3$; $D2_n = 3\text{-}5$; $D3_n = 3$) on the (a) walking, (b) climbing and (c) swimming behaviour of *P. cordiformis*.

		Weight			Damage		
		W1	W2	W3	D1	D2	D3
Walking	P	0.700	0.222	1.00	0.250	0.354	0.400
	U	3.00	3.00	4.00	3.00	2.00	2.00
Climbing	P	0.700	0.786	0.400	0.786	1.00	0.400
	U	3.00	6.00	2.00	6.00	4.00	2.00
Swimming	P	1.00	0.400	0.200	0.250	1.00	1.00
	U	4.00	2.00	1.00	3.00	4.00	4.00

Table. 2.5 P values and test statistics (Mann-Whitney U test) for the effect of (1) sleep deprivation (n= 16) and (2) sleep deprivation delineated by sex (n_{males} = 4-6; $n_{females}$ = 4 on (a) colour, (b) pattern and (c) texture expression in *P. cordiformis*.

		Sleep Deprivation	Sex	
			Male	Female
Colour	P	0.829	1.00	1.00
	U	37.0	12.0	8.00
Pattern	P	0.722	0.914	0.886
	U	35.5	11.0	7.00
Texture	P	0.970	0.914	0.343
	U	50.0	11.0	4.00

Table. 2.6 P values and test statistics (Mann-Whitney U test) for the effect of (1) sleep deprivation delineated by weight ($W1_n$ = 4; $W2_n$ = 7; $W3_n$ = 5), (2) Sleep deprivation delineated by damage ($D1_n$ = 3; $D2_n$ = 3-5; $D3_n$ = 3) on the (a) colour, (b) pattern and (c) texture behaviour in *P. cordiformis*.

		Weight			Damage		
		W1	W2	W3	D1	D2	D3
Colour	P	0.2	0.786	1.00	0.786	0.400	1.00
	U	1.00	6.00	4.00	6.00	2.00	4.00
Pattern	P	0.700	0.881	0.700	0.07	1.00	1.00
	U	1.00	6.50	3.00	3.00	4.00	4.00
Texture	P	0.400	0.250	0.200	0.036	0.700	0.200
	U	2.00	3.00	1.00	0.00	3.00	1.00

Appendix 3: Prey Playback

Table. 3.1 P values and test statistics (Kruskal-Wallis ANOVA) for the effect of (1) prey shape (n= 19) and (2) prey shape delineated by sex (n_{males} = 11; $n_{females}$ = 8) on (a) luring behaviour and (b) alertness of *P. cordiformis* in response to video playback. * indicates a significant effect.

		Shape	Sex	
			Male	Female
Luring	P	0.082	0.648	0.092
	H	6.72	1.65	6.45
Alertness	P	0.110	0.234	0.030*
	H	6.03	4.27	8.95

Table. 3.2 P values and test statistics (Kruskal-Wallis) for the effect of (1) prey shape delineated by weight ($W1_n$ = 4; $W2_n$ = 7; $W3_n$ = 5) and (2) prey motion delineated by damage ($D1_n$ = 3; $D2_n$ = 3-5; $D3_n$ = 3) on (a) luring behaviour and (b) alertness of of *P. cordiformis* in response to video playback. * indicates a significant effect.

		Weight			Damage		
		W1	W2	W3	D1	D2	D3
Luring	P	0.740	0.027*	0.939	0.342	0.024*	0.849
	H	1.27	9.300	0.406	3.35	9.481	0.804
Alertness	P	0.492	0.046*	0.165	0.190	0.0003*	0.099
	H	2.41	7.99	5.10	4.77	19.1	6.28

Table. 3.3 P values and test statistics (Kruskal-Wallis ANOVA) for the effect of (1) prey motion (n= 19) and (2) prey motion delineated by sex (n_{males} = 11; $n_{females}$ = 8) on (a) luring behaviour and (b) alertness of *P. cordiformis* in response to video playback. * indicates a significant effect.

		Motion	Sex	
			Male	Female
Luring	P	0.020*	2.03	3.82
	H	13.4	7.24	5.29
Alertness	P	0.213	0.337	0.063
	H	7.12	5.71	10.5

Table. 3.4 P values and test statistics (Kruskal-Wallis) for the effect of (1) prey motion delineated by weight ($W1_n$ = 4; $W2_n$ = 7; $W3_n$ = 5) and (2) prey motion delineated by damage ($D1_n$ = 3; $D2_n$ = 3-5; $D3_n$ = 3) on (a) luring behaviour and (b) alertness of of *P. cordiformis* in response to video playback.

		Weight			Damage		
		W1	W2	W3	D1	D2	D3
Luring	P	0.334	0.384	0.739	0.468	0.133	0.133
	H	5.72	5.27	2.74	4.59	8.44	8.44
Alertness	P	0.428	0.373	0.320	0.220	0.084	0.132
	H	4.90	5.36	5.87	4.91	9.72	8.47

Table. 3.5 P values and test statistics (Kruskal-Wallis ANOVA) for the effect of (1) prey shape (n= 19) and (2) prey shape delineated by sex (n_{males} = 11; $n_{females}$ = 8) on (a) colour, (b) pattern and (c) texture expression in *P. cordiformis* in response to video playback.

		Shape	Sex	
			Male	Female
Colour	P	0.544	0.003	0.17
	H	2.14	14.3	5.01
Pattern	P	0.119	0.844	0.405
	H	5.86	0.822	2.91
Texture	P	0.535	0.398	0.818
	H	2.19	2.96	0.931

Table. 3.6 P values and test statistics (Kruskal-Wallis) for the effect of (1) prey shape delineated by weight ($W1_n = 4$; $W2_n = 7$; $W3_n = 5$) and (2) prey shape delineated by damage ($D1_n = 3$; $D2_n = 3\text{-}5$; $D3_n = 3$) on (a) colour, (b) pattern and (c) texture expression in *P. cordiformis* in response to video playback.

		Weight			Damage		
		W1	W2	W3	D1	D2	D3
Colour	P	0.139	0.374	0.871	0.833	0.570	0.726
	H	5.50	3.12	0.708	0.366	1.13	0.640
Pattern	P	0.928	0.665	0.328	0.493	0.780	0.529
	H	0.458	1.57	2.95	2.406	1.09	2.21
Texture	P	0.282	0.856	0.598	0.734	0.612	0.753
	H	3.814	0.774	1.88	1.28	1.81	1.20

Table. 3.7 P values and test statistics (Kruskal-Wallis ANOVA) for the effect of (1) prey motion (n= 19) and (2) prey motion delineated by sex (n_{males} = 11; $n_{females}$ = 8) on (a) colour, (b) pattern and (c) texture expression in *P. cordiformis* in response to video playback. * indicates a significant effect.

		Motion	Sex	
			Male	Female
Colour	P	<0.0001*	0.511	0.392
	H	35.2	3.35	5.20
Pattern	P	0.016*	0.816	0.081
	H	13.9	2.24	9.80
Texture	P	0.003	0.315	0.068
	H	18.3	5.35	10.3

Table. 3.8 P values and test statistics (Kruskal-Wallis) for the effect of (1) prey motion delineated by weight ($W1_n$ = 4; $W2_n$ = 7; $W3_n$ = 5) and (2) prey motion delineated by damage ($D1_n$ = 3; $D2_n$ = 3-5; $D3_n$ = 3) on (a) colour, (b) pattern and (c) texture expression in *P. cordiformis* in response to video playback.

		Weight			Damage		
		W1	W2	W3	D1	D2	D3
Colour	P	0.069	0.244	0.897	0.343	0.581	0.704
	H	10.2	6.67	1.64	5.64	3.78	2.97
Pattern	P	0.939	0.788	0.322	0.910	0.682	0.524
	H	1.26	2.43	5.43	1.53	2.88	4.03
Texture	P	0.138	0.434	0.297	0.110	0.574	0.269
	H	8.35	4.62	6.09	8.71	3.83	6.31

Table. 3.9 P values and test statistics (Kruskal-Wallis ANOVA) for the effect of (1) prey shape (n= 19) and (2) prey shape delineated by sex (n_{males} = 11; $n_{females}$ = 8) on (a) peering behaviour and (b) ventilation rates of *P. cordiformis* in response to video playback. * indicates a significant effect.

		Shape	Sex	
			Male	Female
Peering	P	0.733	0.752	0.392
	H	1.28	1.21	4.11
Ventilation	P	0.004*	0.284	0.057
	H	13.4	3.80	7.53

Table. 3.10 P values and test statistics (Kruskal-Wallis) for the effect of (1) prey shape delineated by weight ($W1_n$ = 4; $W2_n$ = 7; $W3_n$ = 5) and (2) prey shape delineated by damage ($D1_n$ = 3; $D2_n$ = 3-5; $D3_n$ = 3) on (a) peering behaviour and (b) ventilation rates of *P. cordiformis* in response to video playback. * indicates a significant effect.

		Weight			Damage		
		W1	W2	W3	D1	D2	D3
Peering	P	0.191	0.324	0.740	0.361	0.368	0.545
	H	4.75	3.48	1.25	3.20	2.00	2.13
Ventilation	P	0.020*	0.664	0.134	0.424	0.543	0.066
	H	9.85	1.58	5.58	2.80	2.14	7.18

Table. 3.11 P values and test statistics (Kruskal-Wallis ANOVA) for the effect of (1) prey motion (n= 19) and (2) prey motion delineated by sex (n_{males} = 11; $n_{females}$ = 8) on (a) peering behaviour and (b) ventilation rates of *P. cordiformis* in response to video playback. * indicates a significant effect.

		Motion	Sex	
			Male	Female
Peering	P	0.0006*	0.806	0.198
	H	21.9	2.30	7.32
Ventilation	P	0.004*	0.355	0.058
	H	17.2	5.53	10.7

Table. 3.12 P values and test statistics (Kruskal-Wallis) for the effect of (1) prey motion delineated by weight ($W1_n$ = 4; $W2_n$ = 7; $W3_n$ = 5) and (2) prey motion delineated by damage ($D1_n$ = 3; $D2_n$ = 3-5; $D3_n$ = 3) on (a) peering behaviour and (b) ventilation rates of *P. cordiformis* in response to video playback.

		Weight			Damage		
		W1	W2	W3	D1	D2	D3
Peering	P	0.121	0.138	0.411	0.545	0.670	0.168
	H	8.72	8.34	4.82	2.13	1.47	7.79
Ventilation	P	0.067	0.531	0.363	0.152	0.566	0.289
	H	10.3	4.13	5.45	8.08	3.88	6.18

Appendix 4: Predator Playback

Table. 4.1 P values and test statistics (Kruskal-Wallis ANOVA) for the effect of (1) predator playback (n= 19) and (2) predator playback delineated by sex (n_{males} = 11; $n_{females}$ = 8) on (a) colour, (b) pattern and (c) texture expression in *P. cordiformis*. * indicates a significant effect.

		Predator	Sex	
			Male	Female
Colour	P	0.967	0.375	0.408
	H	0.567	4.24	3.98
Pattern	P	0.967	1.00	0.939
	H	0.563	0.007	0.798
Texture	P	0.004*	0.025*	0.566
	H	15.3	11.1	2.95

Table. 4.2 P values and test statistics (Kruskal-Wallis) for the effect of (1) predator playback delineated by weight ($W1_n$ = 4; $W2_n$ = 7; $W3_n$ = 5) and (2) predator playback delineated by damage ($D1_n$ = 3; $D2_n$ = 3-5; $D3_n$ = 3) on (a) colour, (b) pattern and (c) texture expression in *P. cordiformis*.

		Weight			Damage		
		W1	W2	W3	D1	D2	D3
Colour	P	0.923	0.631	0.915	0.889	1.00	0.875
	H	0.912	2.58	0.967	1.13	0.000	1.22
Pattern	P	1.000	0.979	1.000	1.000	1.000	1.000
	H	0.038	0.453	0.000	0.018	0.000	0.026
Texture	P	1.000	0.961	0.702	0.608	0.840	0.110
	H	0.038	0.517	2.19	2.71	1.43	7.53